

A. MURRAY, III • D. L. WILLIAMS

ORGANIC SYNTHESSES
WITH
ISOTOPES

PART II

CFTRI-MYSORE



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Organic synthesis

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ORGANIC SYNTHESES WITH ISOTOPES

by Arthur Murray, III
and D. Lloyd Williams

Part I:

Compounds of Isotopic Carbon

Part II:

Organic Compounds Labeled with Isotopes of the Halogens,
Hydrogen, Nitrogen, Oxygen, Phosphorus, and Sulfur



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ARTHUR MURRAY, III, and D. LLOYD WILLIAMS

*University of California, Los Alamos Scientific Laboratory
Los Alamos, New Mexico*

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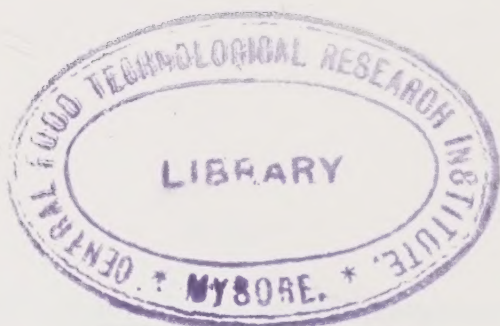
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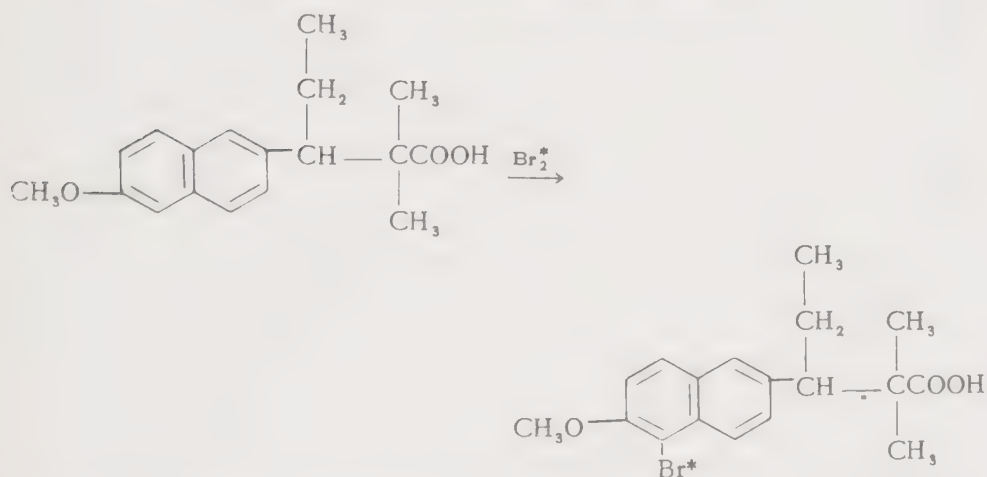
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ISOTOPIC HALOGEN COMPOUNDS

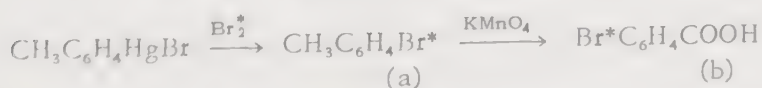
A. BROMINE COMPOUNDS

5-BROMO- β -ETHYL-6-METHOXY- α,α -DIMETHYL-2-NAPHTHALENEPROPIONIC-Br⁸² ACID

P. Sue, J. Jacques, A. Horeau, and R. Courier, *Compt. rend.*, 227, 9 (1948).

Procedure

β -Ethyl-6-methoxy- α,α -dimethyl-2-naphthalenepropionic acid is rapidly brominated in the naphthalene nucleus when treated with bromine in carbon tetrachloride. The bromo compound is recrystallized from aqueous alcohol. Using 2 to 10-mg. quantities of the acid, the reaction gives yields of 80-90% after two recrystallizations of the product, m.p. 185-186°. (The location of the bromine is presumed, but not established.)

2-, 3- AND 4-BROMOBENZOIC-Br⁸² ACIDS

W. J. Klapproth and F. H. Westheimer, *J. Am. Chem. Soc.*, 72, 4461 (1950).

A. Procedure

(a) *2-, 3- and 4-Bromotoluene-Br⁸²*. Tolymercuric bromide, 0.5 g., is stirred with 50 ml. of chloroform (Note 1). The chloroform solution of bromine isotope (Note 2) is added in small portions until the brown color of bromine persists; then the mixture is stirred for 2 hours. The excess bromine is extracted with sodium bisulfite solution, and the mercuric bromide formed is extracted with aqueous sodium bromide solution. The chloroform solution is washed with water and divided into three 100-ml. portions. Into three separate flasks, 10 g. of each of the pure isomeric bromotoluenes is weighed. One of the 100-ml. portions of labeled tracer mixture is added to each flask (Note 3). The chloroform is distilled from each sample through a short column, and last traces are removed by steam distillation with added water.

(b) *2-, 3- and 4-Bromobenzoic-Br⁸² Acid*. Each residue of bromotoluene from the steam distillation is oxidized with alkaline permanganate.¹ The following procedure for the purification of the isomeric bromobenzoic acids is carried out concurrently over a period of two days.

(1) *2-Bromobenzoic-Br⁸² Acid*. The manganese dioxide is removed by filtration, and the filtrate is evaporated to 400 ml. The hot solution is acidified with 6 *N* hydrochloric acid and treated twice with 1-g. portions of charcoal, and the cooled filtrate is extracted with ether. The ether is evaporated, and the residue is recrystallized twice from carbon tetrachloride-absolute alcohol (5:1) (Note 4). The product from the second recrystallization is weighed and diluted with enough pure inactive 2-bromobenzoic acid to make a total of 3 g. The isotopically diluted acid is then recrystallized from the carbon tetrachloride-ethanol mixture, and the activity is determined. Recrystallization is repeated until there is no further change in the counting rate.

(2) *3-Bromobenzoic-Br⁸² Acid*. The manganese dioxide is filtered from the oxidation mixture containing the added *m*-isomer, and the filtrate is acidified and cooled. The impure 3-bromo acid is collected, dried and dissolved in 600 ml. of hot 50% ethanol, and 10 g. of inactive 4-bromobenzoic acid is added (Note 5). The precipitate of *p*-acid is filtered from the slowly cooled solution, which is then concentrated to 300 ml. Barium hydroxide is added, and the resulting barium salt¹ is recrystallized from water. The *m*-acid recovered from the barium salt upon acidification is recrystallized from 50% methanol. The 0.5 to 1.5 g. of *m*-acid so obtained is diluted with pure, inactive *m*-acid to 4 g. and treated twice in hot 20% ethanol solution with 1-g. portions of carbon. The acid, which crystallizes from the cooled solution, is recrystallized from 25% methanol to constant specific activity (Note 6).

(3) 4-Bromobenzoic-Br⁸² Acid. The filtrate, from manganese dioxide removal, is acidified and filtered while hot. The *p*-acid, obtained on cooling the solution, is twice recrystallized from 50% ethanol

B. Notes

1. Toluene is mercurated with mercuric perchlorate or mercuric acetate. The ratio of *o*-, *m*- and *p*- substitution is greatly affected by the choice of reagent and the temperature of the reaction. In the procedure of Coffey,² toluene is heated under reflux with mercuric acetate for 1.5 hours; the percentages of *o*-, *m*- and *p*- isomers resulting are respectively 43, 13 and 44 with about 15% of dimercuration. The mixture of isomeric tolymercuric bromides is precipitated by the addition of sodium bromide solution, after removal of excess mercuric acetate and toluene; yield, 25.6%.

2. Irradiated potassium bromide (0.9 g.) is dissolved in water (50 ml.). Portions of the solution are withdrawn as needed and shaken with a solution of bromine in chloroform; most of the activity is extracted into the chloroform layer.

3. The weight of tracer is small compared to the weight of carrier.

4. In the original work, only 50-75% of the material was recovered each time, purposely.

5. This serves as a "hold back" carrier.

6. This complex procedure was required for the *m*-acid because the *p*-acid showed a strong tendency to coprecipitate.

C. Other Preparations

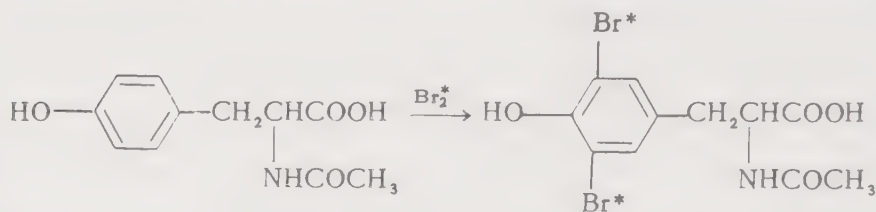
2-, 3- and 4-Bromobenzoic-Br⁸² acids have been prepared by Ivanoff.³ A mixture of ethylene bromide and methyl or ethyl benzoate was irradiated with neutrons. The resulting mixture of 2-, 3- and 4-bromobenzoate esters was hydrolyzed with alcoholic potassium hydroxide. After evaporation of the alcohol and acidification of the residue with hydrochloric acid, 3- and 4-bromobenzoic-Br⁸² acids were steam-distilled. The 2-bromobenzoic-Br⁸² acid in the residue from steam distillation was extracted with ether, dried and converted to ammonium 2-bromobenzoate-Br⁸² with dry ammonia. From the steam-distillate, the insoluble *p*-isomer was collected by filtration and recrystallized from chloroform. The *m*-isomer, obtained by partial evaporation of the filtrate, was dissolved in ether, dried and converted to ammonium 3-bromobenzoate-Br⁸² with dry ammonia.

¹L. A. Bigelow, J. Am. Chem. Soc., 44, 2010 (1922).

²S. Coffey, J. Chem. Soc., 127, 1029 (1925).

³M. Ivanoff, Bull. soc. chim. France, 1953, 266.

***N*-ACETYL-3,5-DIBROMO-L-TYROSINE-Br⁸²₂**
[2-Acetamido-3-(3,5-dibromo-4-hydroxyphenyl)propionic-Br⁸²₂ Acid]



D. G. Doherty and F. Vaslow, J. Am. Chem. Soc., 74, 931 (1952).

A. Procedure

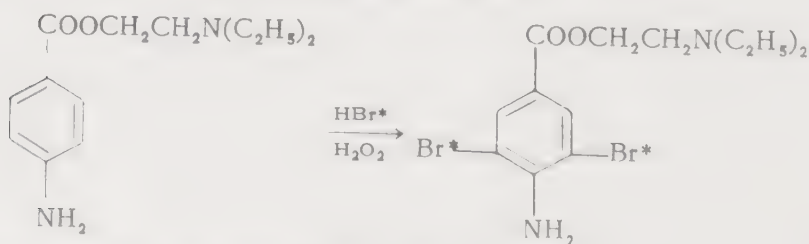
Bromine-Br⁸²₂ is entrained with air and bubbled through a solution of 70 mg. of *N*-acetyl-L-tyrosine in 1 ml. of glacial acetic acid. The solution is heated in a steam-bath for 1 minute and then evaporated to dryness *in vacuo*. The product is crystallized from water (Notes 1 and 2).

B. Notes

1. This product was further purified by dissolution in 5 ml. of 0.04 *N* sodium hydroxide-0.25 *N* sodium chloride solution and absorption on a 1 × 5-cm. Dowex-1 column in equilibrium with the same solvent. Elution was effected with the same solvent, and 200 ml. of the peak of the eluate was acidified and extracted with ethyl acetate. The extract was washed with water, re-extracted into an aqueous sodium bicarbonate solution and used experimentally.

2. In a similar preliminary experiment using 20 g. of *N*-acetyl-L-tyrosine, the yield of crude brominated product, m.p. 115°, was 26.1 g. The compound, soluble in alcohol, dioxane, ethyl acetate and acetone, was recrystallized from aqueous alcohol to constant melting point, 123-123.5°.

DIETHYLAMINOETHYL 4-AMINO-3,5-DIBROMOBENZOATE-Br⁸²₂
(Dibromoprocaine-Br⁸²₂)



F. Howarth, Nature, 161, 857 (1948); J. Pharmacol., 4, 333 (1949).

A. Procedure

Procaine is brominated according to the procedure of Morel, *et al.*¹ (Note 1). To a concentrated solution of hydrobromic-Br⁸² acid (from 0.5 g. of silver bromide-Br⁸² is added the calculated amount of procaine and about 0.5 ml. of hydrogen peroxide (30%). There is a brisk reaction and rapid precipitation of dibromoprocaine-Br⁸² (Note 2).

In the later reference, the exact amount of procaine is not determined. To the concentrated hydrobromic acid is added 3-4 ml. of 30% hydrogen peroxide, and the bromination is then controlled by addition of procaine in small amounts until the solution becomes colorless. The dibromoprocaine-Br⁸² hydrobromide, thus obtained, is converted to the hydrochloride by precipitation of the free base with 0.1 N sodium hydroxide and treatment of its ether solution with hydrogen chloride.

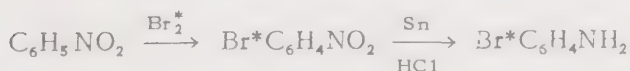
B. Notes

1. Morel, *et al.* ascribe the 3,5-dibromo configuration by analogy with the bromination of *p*-aminobenzoic acid.

2. If the reaction becomes too violent it is slowed by the addition of more peroxide. The time taken for the reaction may vary from minutes to hours depending upon the concentration of reagents. Since the half-life of bromine-82 is 34 hours, a high concentration of hydrobromic-Br⁸² acid is desirable.

¹A. Morel, A. Leulier and P. Denoyel, *Bull. soc. chim. France*, 45, 457 (1929).

4-BROMOANILINE-Br⁸²



M. Ivanoff, *Bull. soc. chim. France*, 1953, 266.

A. Procedure

(a) 1-Bromo-2-, 3- and 4-nitrobenzene-Br⁸². A mixture of equal volumes (5-10 ml.) of nitrobenzene and ethylene bromide is exposed to neutron bombardment. Free bromine is then removed from the mixture by treatment with sodium sulfite solution. The mixture of bromonitrobenzene isomers is then distilled at 10-20 mm., boiling range 130-135°.

(b) 2-, 3- and 4-Bromoaniline-Br⁸². The above mixture of isomeric bromonitrobenzenes is reduced with a boiling mixture of tin and hydrochloric acid during 30 minutes (Note 1). The solution is then made strongly basic with sodium hydroxide, and the mixture of 2-, 3- and 4-bromoaniline-Br⁸² is isolated by steam distillation.

(c) 2-, 3- and 4-Bromoaniline-Br⁸² Hydrochloride. The cooled steam-distillate is extracted with ether which is combined and dried. The dry ether solution of isomeric bromoanilines is then saturated with dry hydrogen chloride. The mixture of isomeric bromoaniline-Br⁸² hydrochlorides is recrystallized from a mixture of alcohol and ether.

(d) 2-Bromoaniline-Br⁸². The mixture of 2-, 3- and 4-bromoaniline-Br⁸² hydrochlorides is dissolved in water, acidified with a few drops of concentrated hydrochloric acid, and subjected to steam distillation. The distillate is cooled and extracted with ether. The ether extract of 2-bromoaniline-Br⁸² is concentrated and treated with an alcoholic solution of picric acid. The resulting 2-bromoaniline-Br⁸² picrate is recrystallized several times from alcohol.

(e) 3- and 4-Bromoaniline-Br⁸². The residual solution from the above steam distillation, containing 3- and 4-bromoaniline-Br⁸², is made basic with sodium hydroxide, and steam distillation is continued. The resulting mixture of 3- and 4-bromoaniline-Br⁸² is separated by making use of differences in the solubilities of their picrates and phosphates in ethyl and methyl alcohols (Note 2).

B. Notes

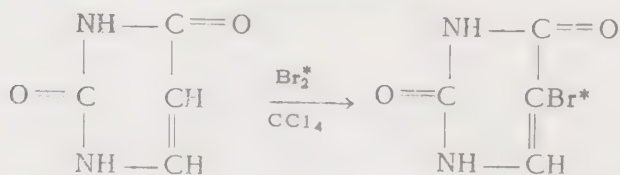
1. For the reduction of nitro compounds with tin and hydrochloric acid see Clarke¹ and Buck.²

2. Using the above labeled isomers it was established that no isotopic exchange occurs during vacuum distillation, reduction of the nitro compounds with tin and hydrochloric acid or steam distillation.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 455.

²*Ibid.*, Coll. Vol. II, Wiley, New York, 1943, p. 130.

5-BROMOURACIL-Br⁸²

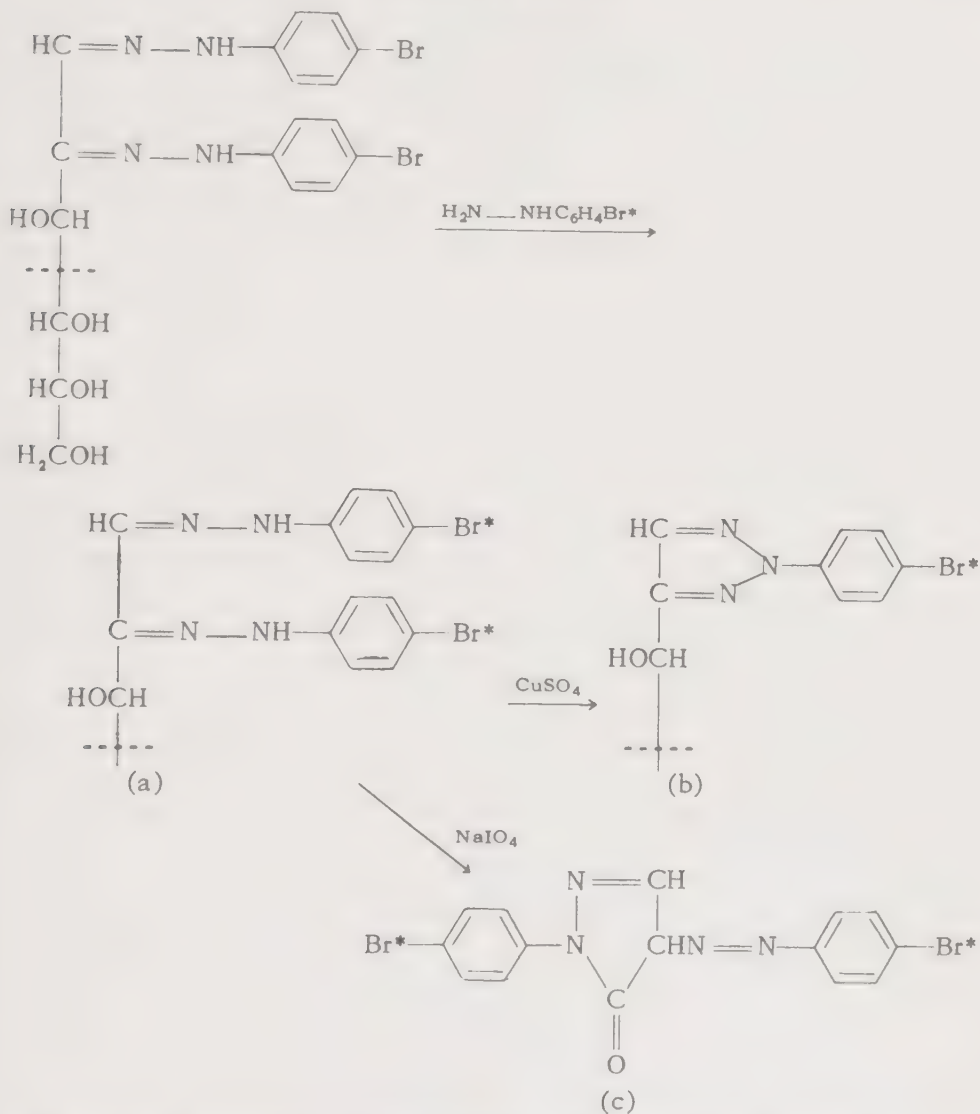


F. Weygand, A. Wacker and H. Grisebach, *Z. Naturforsch.*, **6b**, 177 (1951).

Procedure

A suspension of 250 mg. of uracil in 10 ml. of carbon tetrachloride containing 380 mg. (0.12 ml.) of bromine-Br⁸² is refluxed for 10 hours. After removal of the solvent *in vacuo*, the residue is twice recrystallized from hot water. The yield of product, m.p. 300°, is 168 mg.

1-(4-BROMOPHENYL)-4-(4-BROMOPHENYLAZO)-2-PYRAZOLIN-5-ONE-Br₂⁸²



F. Weygand, H. Grisebach and K. Schmeiser, *Angew. Chem.*, 63, 27 (1951).

A. Procedure

(a) *D*-Glucose Bis(4-bromophenyl)osazone-Br₂⁸². 4-Bromophenyldiazine-Br⁸² is heated for 2 hours under reflux in aqueous dioxane (pH adjusted to 4.2 with acetic acid) with *D*-glucose bis(4-bromophenyl)osazone. About 22% of the radioactive 4-bromophenyldiazine exchanges with the inactive material (Note 1).

(b) *D-Glucose 4-Bromophenylosotriazole-Br⁸²*. The *D*-glucose bis(4-bromophenyl)osazone-Br⁸² is heated with copper sulfate in aqueous 2-propanol according to the general procedure of Hann and Hudson¹ to obtain the *D*-glucose 4-bromophenylosotriazole-Br⁸² (Note 2). In addition, 4-bromoaniline-Br⁸², which is converted to *N*-(4-bromophenyl)benzamide-Br⁸² for radioactivity assay, is also obtained as by-product.

(c) *1-(4-Bromophenyl)-4-(4-bromophenylazo)-2-pyrazolin-5-one-Br₂⁸²*. The osazone (a) is oxidized^{2,3} with periodic acid to obtain the pyrazolone. Reduction of the latter with zinc chloride and hydrochloric acid gives one mole of 4-bromoaniline-Br⁸² which is converted to the benzamido derivative (Note 3).

B. Notes

1. The rate of exchange of 4-bromophenylhydrazine residues at C-1 and C-2 is quite different. Radioactivity measurements on the degradation products indicate 75% of the activity at C-1 and 25% at C-2.

2. This general reaction has been applied to a number of sugars.^{4,5}

3. Radioactivity measurements on the various products indicated that the 4-bromoaniline-Br⁸², eliminated by the action of copper sulfate on (a), is from the 4-bromophenylhydrazine residue attached to C-1. The 4-bromoaniline-Br⁸² obtained by reduction of the pyrazolone came from the phenylhydrazine residue at C-2 of the glucosazone molecule.

¹R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **66**, 735 (1944).

²P. Karrer and K. Pfähler, *Helv. Chim. Acta*, **17**, 766 (1934).

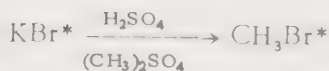
³E. Chargaff and B. Magasanik, *J. Am. Chem. Soc.*, **69**, 1469 (1947).

⁴W. T. Haskins, R. M. Hann and C. S. Hudson, *J. Am. Chem. Soc.*, **67**, 939 (1945); **68**, 1766 (1946); **69**, 1050, 1461 (1947).

⁵C. S. Hudson, *J. Org. Chem.*, **9**, 470 (1944).

BROMOMETHANE-Br⁸²

METHOD I



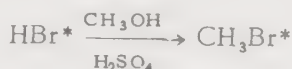
C. Baret and L. Pichat, *Bull. soc. chim. France*, 1950, 1294.

A. Procedure

To a solution of potassium bromide-Br⁸² in a round-bottomed flask is added 0.5 ml. of concentrated sulfuric acid and 3.5 ml. of methyl sulfate. When the flask is heated to 60–70°, bromomethane-Br⁸² is formed and and passed successively through a water-cooled condenser, a wash

bottle containing water, another containing sulfuric acid, and a potassium hydroxide tube. The bromomethane-Br⁸² is collected in a trap cooled to -70°. After the mixture is heated for 5 minutes, the remainder of the product is swept into the trap with a stream of air. The colorless bromomethane-Br⁸² distills from the trap at 5.5° without leaving a residue; yield, 96%. The entire synthesis requires about 25 minutes.

METHOD II



F. P. W. Winteringham, J. Chem. Soc., 1949, 416.

A. Procedure

Hydrogen bromide-Br⁸² is vacuum-distilled into a 20-ml. reaction flask equipped with a twin by-pass funnel which permits introduction of two reagents independently. From the first funnel, aqueous methanol is added on top of the frozen hydrogen bromide-Br⁸² which dissolved immediately. Concentrated sulfuric acid is gradually added from the second funnel, and the reaction mixture is gently heated (Note 1). The methyl bromide-Br⁸² passes through a water-cooled condenser and a purifying train, consisting of a long tube packed successively with cotton saturated with water, soda lime and anhydrous calcium chloride, and is finally collected at -180° in a U-tube (Note 2).

B. Notes

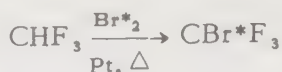
1. The relative weights of the reagents used were based on the optimum values determined by Bygden,¹ who actually used potassium bromide instead of hydrogen bromide and obtained yields of 95-97%. The molar ratios of the reactants: sulfuric acid, water, methanol and potassium bromide were, respectively, 3:9:3:1.

2. The boiling point of the product may be checked by isolating and evacuating the tube containing the frozen sample. The temperature at which the vapor pressure of the sample reaches 76 cm. is then determined.

C. Other Preparations

Methyl bromide-Br⁸² has also been prepared by isotopic exchange between metal bromides-Br⁸² and inactive methyl bromide. See Halogen Exchange Reactions, Table XV, 1.

¹A. Bygden, J. prakt. Chem., 104, 285 (1922).

BROMOTRIFLUOROMETHANE-Br⁸⁰

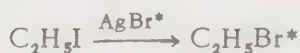
R. S. H. Chiang and J. E. Willard, *J. Am. Chem. Soc.*, **74**, 6214 (1952).

A. Procedure

Bromotrifluoromethane is prepared by passing a mixture of fluoroform and bromine-Br₂⁸⁰ back and forth over a red hot spiral of platinum wire until the bromine color disappears (Note 1). The hydrogen bromide formed in the reaction is removed by passing the product mixture through a sodium hydroxide train (Note 2).

B. Notes

1. All the operations were carried out in a vacuum system.
2. The excess fluoroform was not removed.

BROMOETHANE-Br⁸⁰

P. Sue and J. Beydon, *Bull. soc. chim. France*, **11**, 56 (1944).

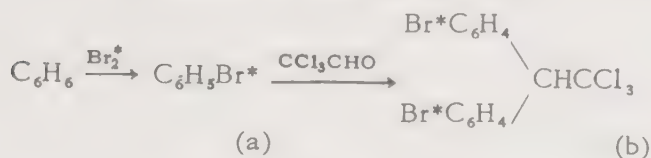
A. Procedure (Note 1)

Into a copper flask with a long neck is introduced the silver bromide, a large excess of ethyl iodide and a few drops of alcohol (90%). The neck is sealed, and the flask is agitated in a boiling water-bath. After 5-10 minutes the reaction is complete; the flask is cooled and opened, a small column is attached, and the product is separated by distillation. The bromoethane-Br⁸⁰ is collected in a tube cooled with a suitable bath. Although the mass yields are of the order of 96.5-100%, the radioactivity yields are from 45% to 54% (Note 2).

B. Notes

1. In the reaction of ethyl iodide with isotopic silver bromide it is possible to use a quantity of silver bromide equivalent to 1 mg. of bromine and complete the reaction in 40 minutes. Because of the short half-lives of bromine and iodine isotopes rapid procedures are desirable.
2. The isotopic yield is based on the measured activity of the silver bromide used and the ethyl bromide prepared. On the 1-mg. scale, only a radioactivity yield determination was possible (54%).

2,2-BIS(4-BROMOPHENYL)-1,1,1-TRICHLOROETHANE-Br⁸²
(Bromine-82 Analog of DDT)



F. P. Winteringham, A. Harrison, C. R. Jones, J. L. McGirr and W. H. Templeton, *J. Sci. Food Agr.*, **1**, 214 (1950).

A. Procedure

(a) *Bromobenzene-Br⁸²*. Using a vacuum apparatus (Figure XV, 1), the bromine-Br⁸², prepared from 700 mg. of silver bromide-Br⁸², 1.5 ml. of concentrated sulfuric acid and 400 mg. of potassium dichromate, is distilled into a reaction tube cooled to -180° and containing 0.5 ml. of dry benzene and 50 mg. of fine iron filings. Dry air is admitted, and the bromination is allowed to proceed for 2 hours at a final temperature of 65° . The evolved hydrogen bromide-Br⁸² is collected in the bromine generator cooled to -180° (Note 1). After the reaction period, the reactor tube is again cooled with liquid air and evacuated. Then the

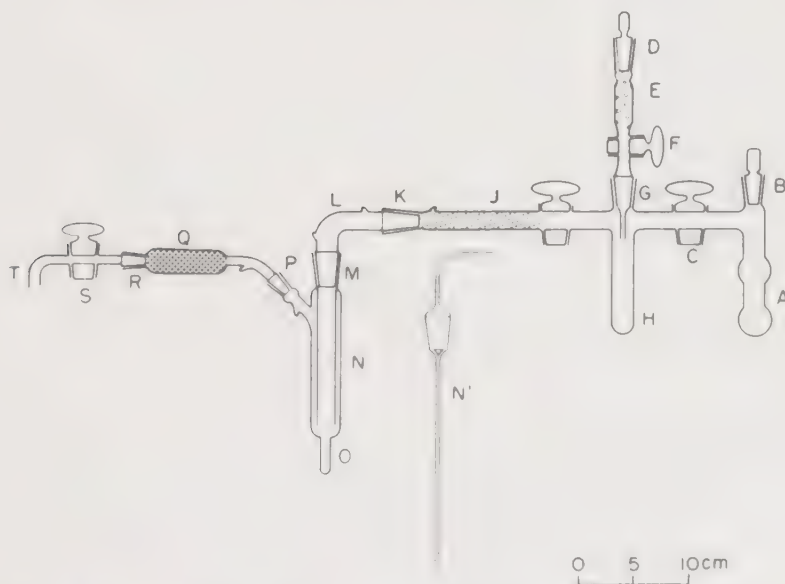


Fig. XV, 1 Vacuum apparatus for the preparation of the bromine-Br⁸² analog of DDT (F. P. Winteringham, A. Harrison, C. R. Jones, J. L. McGirr and W. H. Templeton). A, bromine-Br⁸² generator containing sulfuric acid; B, stopper for addition of silver bromide-Br⁸² and potassium dichromate; E, calcium chloride; H, reaction tube containing dry benzene and iron filings; J, soda lime; N, trap at -180° for collecting bromobenzene-Br⁸² and unreacted benzene; O, graduated tube; N', tube for introducing nitrogen into O; Q, activated charcoal.

bromobenzene-Br⁸²-benzene mixture is vacuum distilled through a tube containing soda lime into a reaction tube with a small graduated lower portion. Dry air is admitted to the apparatus, and by means of a ground joint at the top of the tube an inlet tube is added with a capillary reaching to the graduated portion of the reaction tube. The benzene solution is evaporated to about 0.12 to 0.13 ml. with a stream of nitrogen heated to 150°. The residual fraction is nearly pure bromobenzene-Br⁸² in about 60% yield.

(b) *2,2-Bis(4-bromophenyl)-1,1,1-trichloroethane-Br₂⁸²*. The inlet tube is removed, and 0.1 g. of chloral hydrate and 1.0 ml. of concentrated sulfuric acid are added to the bromobenzene-Br⁸². The tube is stoppered, briefly heated to 100° and then allowed to cool while being mechanically shaken. When the reaction mixture is poured onto crushed ice, the crude product separates as a gummy solid. The product is washed with sodium bicarbonate solution and crystallized from hot ethanol. The final product, in 15% yield, melts at 141–143° (Note 2). The two bromine atoms are quite stably bound (Note 3).

B. Notes

1. No attempt was made to reoxidize the hydrogen bromide-Br⁸² collected.

2. Attempts to improve the yield on this scale were unsuccessful.

3. Neither of the two bromine atoms was affected by heating the product with a solution of sodium in ethanolamine, in a sealed tube, long enough to remove all three of the chlorine atoms (5 hours at 150°). No exchange with barium bromide was detected after 18 hours at room temperature in methanol-acetone mixture.

C. Other Preparations

The bromine-82 analog of DDT has also been prepared¹ by a similar procedure.

Bromobenzene-Br⁸² and -Br⁸⁰ have been prepared,² together with bromonaphthalene-Br⁸² and -Br⁸⁰, in a comparative reaction rate study. A mixture of benzene and naphthalene was treated with a solution of bromine-Br₂⁸² and -Br₂⁸⁰ in ethyl bromide. The brominated components of the mixture were then separated by the isotopic dilution method. The ratio of rates of bromination of naphthalene and benzene was approximately 7. Bromobenzene-Br⁸² has also been prepared in 64–67% yield by the bromination of benzene in the presence of 30% sulfuric acid and an aqueous solution of potassium bromate.³

¹E. L. Hansen, J. W. Hansen and R. Craig, J. Econ. Entomol., 37, 853 (1944).

²S. May, M. Roux, Ng. Ph. Buu-Hoi and R. R. Daudel, Compt. rend., 228, 1865 (1949).

³J. D. Roberts, J. K. Sanford, F. L. J. Sixma, H. Cerfontain and R. Zagt, J. Am. Chem. Soc., 76, 4525 (1954).

BROMOTRIPHENYLETHYLENE-Br⁸²



G. H. Twombly, E. F. Schoenewaldt and D. Meisel, Cancer Research, 11, 780 (1951).

A. Procedure

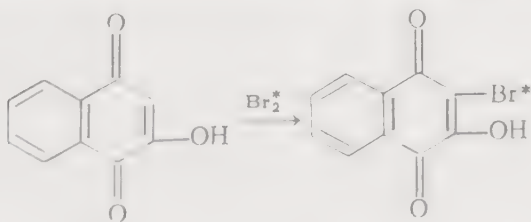
Bromine-⁸²Br₂ is carried, with a slow stream of nitrogen, through a drying trap and into a cold solution of 3–5 mg. of triphenylethylene in freshly distilled chloroform (Note 1). The product is obtained by evaporation of the chloroform solution. The residue is recrystallized from glacial acetic acid to obtain white needles of bromotriphenylethylene-Br⁸², m.p. 114.5–115°.

B. Notes

1. Apelgot¹ also prepared bromotriphenylethylene-Br⁸²; however, the product contained 20% unreacted triphenylethylene, as the result of insufficient bromine-Br⁸² in the reaction. It was found in preliminary experiments, on a small scale (less than 10 mg.), that 2,2-diphenylacetophenone, m.p. 137°, was the predominant product unless the reaction mixture was heated. A 98% yield of bromotriphenylethylene, m.p. 115°, was obtained when 8 mg. of triphenylethylene was added to 6.5 mg. of bromine in warm chloroform and refluxed for 30 minutes. The chloroform solution was treated with 1.5 ml. of 1% alcoholic silver nitrate, centrifuged, separated from the precipitate, washed with water and evaporated in a stream of air. The product was recrystallized from 0.4 ml. of absolute alcohol.

¹S. Apelgot, A. Cheutin, S. Mars and M. R. Berger, Bull. soc. chim. France, 19, 533 (1952).

2-BROMO-3-HYDROXY-1,4-NAPHTHOQUINONE-Br⁸²



M. Berger, Ng. Ph. Buu-Hoi, P. Daudel, R. Daudel and S. May, Experientia, 2, 184 (1946).

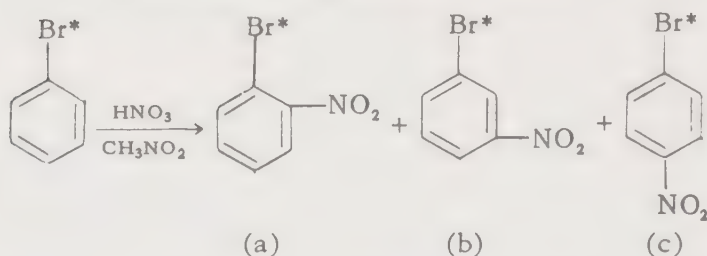
A. Procedure

2-Bromo-3-hydroxy-1,4-naphthoquinone-Br⁸² is prepared by the bromination of 2-hydroxy-1,4-naphthoquinone in boiling acetic acid solution (Note 1).

B. Notes

1. Fries¹ has reported the preparation of 2-bromo-3-hydroxy-1,4-naphthoquinone by heating 2-bromo-1,4-naphthoquinone for a short time with a mixture of alcohol and concentrated hydrochloric acid, followed by treatment with dilute sodium hydroxide. After recrystallization from alcohol the yellow prisms melt at 202°. Solubility in 95% ethanol is 2% at 25°; solubility in water, ether and benzene is slight.

¹K. Fries and K. Schimmelschmidt, *Ann.*, 484, 245 (1930).

1-BROMO-4-NITROBENZENE-Br⁸²

J. D. Roberts, J. K. Sanford, F. L. J. Sixma, H. Cerfontain and R. Zagt, *J. Am. Chem. Soc.*, 76, 4525 (1954).

A. Procedure

Nitration of Bromobenzene-Br⁸². In a typical experiment, 12 g. of bromobenzene-Br⁸², dissolved in 50 ml. of nitromethane, is nitrated at 25° for 11 hours with a mixture of 43 ml. of anhydrous nitric acid in 56 ml. of nitromethane. The reaction mixture is divided into three portions.

(a) *1-Bromo-2-nitrobenzene-Br⁸².* To 12.568 g. of the nitration mixture is added 9.862 g. of *p*-isomer and 10.053 g. of *o*-isomer. The resulting mixture is dissolved in ether, washed with sodium hydroxide solution and water, and dried. After removal of ether, the residue is dissolved in 50 ml. of hot ethanol. The solution is cooled to effect crystallization of a major part of the *p*-isomer. The mother liquor, containing the *o*-isomer, is twice scavenged by addition and recrystallization of inactive *p*-isomer (Note 1). 1-Bromo-2-nitrobenzene-Br⁸² is isolated from the mother liquor by fractional crystallization and recrystallized several times from petroleum ether; m.p. 35.4–36.4° (Note 2).

(b) *1-Bromo-3-nitrobenzene-Br⁸².* Another portion of the nitration mixture, 178.22 g., is added to a solution of 9.151 g. of *m*-isomer and 6.044

g. of *o*-isomer in ether. The resulting solution is neutralized, washed with water and dried. After ether and nitromethane are removed *in vacuo*, the residue is dissolved in 50 ml. of hot ethanol. 1-Bromo-4-nitrobenzene-Br⁸² is removed by repeated crystallization from ethanol, each time with the addition of 9 g. of inactive *p*-isomer.

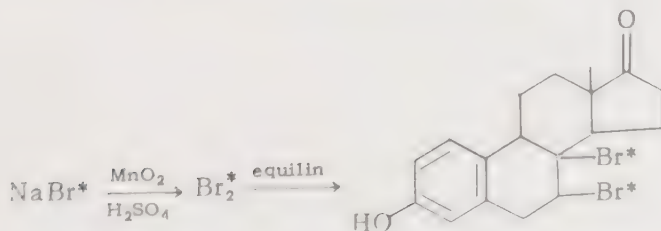
After removal of solvent from the mother liquor, the residue is heated under reflux for 15 minutes with 40 ml. of piperidine. The mixture is cooled, diluted with water and extracted with ether. The ether extract is washed with dilute hydrochloric acid, dried and concentrated. The foregoing piperidine treatment is repeated 3 times, with a few grams of inactive *o*-isomer added each time (Note 3). The resulting 1-bromo-3-nitrobenzene-Br⁸² is recrystallized from petroleum ether, m.p. 52.0–53.0°.

(c) 1-Bromo-4-nitrobenzene-Br⁸². To 12.517 g. of the nitration mixture is added 4.992 g. of *p*-isomer and 3.088 g. of *o*-isomer. After neutralization with sodium hydroxide, the mixture is dissolved in boiling acetic acid. The 1-bromo-4-nitrobenzene-Br⁸², which crystallized upon cooling the solution, is recrystallized twice (Note 4). After the *p*-isomer is again scavenged with added *m*- and *o*-bromonitrobenzene and bromobenzene by recrystallization, it melts at 124.5–125.5° (Note 5).

B. Notes

1. The activity of the last recovered *p*-isomer is negligible.
2. The *o*-isomer still contained a small amount of inactive *p*-isomer.
3. The piperidine hydrobromide from the last treatment was converted to silver bromide and counted. The low activity indicated the scavenging to be satisfactory.
4. The labeled *p*-isomer separated in the isolation of labeled *o*-isomer could be included in the purification of labeled *p*-isomer at this stage, if desired.
5. The distribution of isomers found in the nitration of bromobenzene-Br⁸² was: *ortho*, 36.5%; *meta*, 1.2%; *para*, 62.3%.

7,8-DIBROMOESTRONE-Br₂⁸²



A. Procedure

(a) *Bromine-Br⁸²*. Sodium bromide-Br⁸², 2 to 4 mg., in 3 to 5 ml. of water, is added to an oxidizing mixture consisting of 1 g. of manganese dioxide and 3 ml. of concentrated sulfuric acid. The flask containing the mixture is heated gently with a microburner, and the bromine generated is carried, with a slow stream of nitrogen, into cold chloroform.

(b) *7,8-Dibromoestrone-Br⁸²*. In this case the bromine is dissolved in a cold solution of equilin in 20 ml. of chloroform (Note 1). Most of the bromine is swept over in 1 to 1-1/2 hours. The chloroform solution is separated from a small amount of water, entrained during the distillation, washed twice with 10-ml. portions of water (Note 2), and evaporated to dryness. Slow evaporation results in the formation of rosettes of colorless crystals of 7,8-dibromoestrone-Br⁸² (Note 3).

B. Notes

1. The amount of equilin is 10 to 20% in excess of that theoretically required to combine with the bromine.

2. The solution is washed to remove any hydrobromic acid present. Practically all the activity remains in the chloroform layer.

3. Assay of the product indicated bromination of 83% of the equilin initially taken.

C. Other Preparations

Bromine-Br⁸² has been prepared: from potassium bromide-Br⁸² by the action of a 50% excess of potassium bromate and phosphoric acid;¹ in chloroform solution by an exchange procedure with irradiated potassium bromide;² by the action of hydrogen peroxide on concentrated hydrobromic-Br⁸² acid;³ and by the action of manganese dioxide and concentrated sulfuric acid on sodium bromide-Br⁸².⁴

7,8-Dibromoestrone-Br⁸² has been prepared by the bromination of equilin in chloroform under anhydrous conditions.⁴

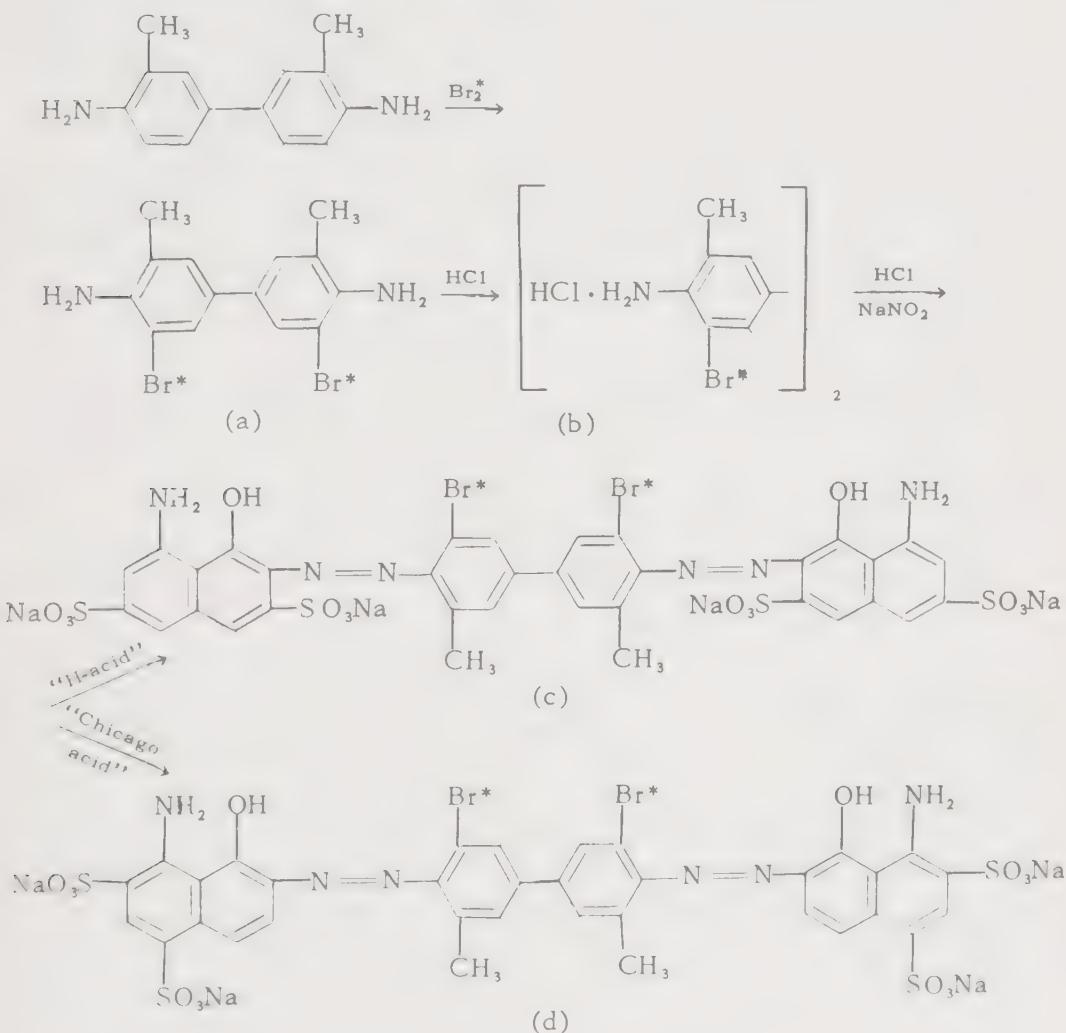
¹J. B. Peri and F. Daniels, J. Am. Chem. Soc., 72, 424 (1950).

²W. J. Klapproth and F. H. Westheimer, J. Am. Chem. Soc., 72, 4461 (1950).

³F. Howarth, Nature, 161, 857 (1948).

⁴G. H. Twombly and E. F. Schoenewaldt, Cancer, 3, 601 (1950).

SODIUM 2,2'-(3,3'-DIBROMO-5,5'-DIMETHYL-4,4'-BIPHENYLENE-BISAZO)BIS(8-AMINO-1-NAPHTHOL-3,6-DISULFONATE)-Br₂⁸²
(Dibromotrypan-Br₂⁸² Blue)



L. H. Tobin and F. D. Moore, J. Clin. Invest., 22, 155 (1943).

A. Procedure

(a) 5,5'-Dibromo-o-tolidine-Br₂⁸², (5,5'-Dibromo-3,3'-dimethylbenzidine-Br₂⁸²). A solution of 1 g. of 3,3'-dimethylbenzidine in 75 to 100 ml. of glacial acetic acid is placed in a 1-l. three-necked flask. The flask is fitted with a stirrer, a dropping funnel and a tube leading to a gas trap. A solution of 1.0 to 1.5 g. of bromine-Br₂⁸² in glacial acetic acid is added slowly from the dropping funnel into the benzidine solution, with stirring (Note 1). When all the bromine is added, stirring is continued for 1 hour.

Then the flask is heated to 85° in a water-bath to complete the reaction. The resultant suspension is cooled, sufficient stannous chloride is added slowly with stirring to reduce excess bromine to bromide, and the green suspension yields a white crystalline precipitate. The volume is made up to 500 ml. with water, and the mixture is cooled for 2 hours. The 5,5'-dibromo-3,3'-dimethylbenzidine- Br_2^{82} is collected on a filter, washed with 50% acetic acid, then with water, and dried. The product is a grayish powder with a melting point of $195\text{--}195.5^{\circ}$; yield 85-90% (Note 2).

(b) *5,5'-Dibromo-o-tolidine- Br_2^{82} Dihydrochloride*. The dry dibromo-o-tolidine- Br_2^{82} is dissolved in toluene. Dry hydrogen chloride is bubbled through the solution to complete precipitation. The precipitate is collected, washed with toluene and ether and dried. The yield of amine hydrochloride is 90%.

(c) *Sodium 2,2'-(3,3'-Dibromo-5,5'-dimethyl-4,4'-biphenylenebisazo)bis(8-amino-1-naphthol-3,6-disulfonate)- Br_2^{82} , (Dibromotrypan- Br_2^{82} Blue)*. The benzidine hydrochloride is suspended in 150 ml. of water, containing 4 equivalents of hydrochloric acid, and cooled to $0\text{--}15^{\circ}$ with stirring. A solution of sodium nitrite, slightly in excess, is run in rapidly (Note 3). A deep greenish-blue color appears as the diazonium salt is formed. The solution is stirred for one-half hour; then, about 1 g. of urea is added to decompose excess nitrous acid.

In a beaker, a paste is made of two equivalents of 8-amino-1-naphthol-3,6-disulfonic acid ("H-Acid") in about 50 ml. of water. Solution of the "H-Acid" is effected by adding 1 equivalent of sodium hydroxide in 10 ml. of water (Note 4). The solution is cooled below 15° , and 4.0 g. of anhydrous sodium carbonate is added to make the solution alkaline. With vigorous stirring, the solution of diazonium salt is run in rapidly (Note 5). After it is stirred for one-half hour, the solution is tested for alkalinity, and more sodium carbonate is added if necessary.

The dye is precipitated by adding 60 grams of sodium acetate for each 100 ml. of the dye solution, heating to 85° , and centrifuging the hot solution. The precipitate is dried overnight at 100° and washed free of acetate with small amounts of hot 95% alcohol.

In an alternate method, the dye solution is concentrated to 50-100 ml., absolute alcohol is added to a concentration of 90%, and the sparingly soluble dye is centrifuged and dried at 100° .

(d) *Sodium 2,2'-(3,3'-Dibromo-5,5'-dimethyl-4,4'-biphenylenebisazo)bis(8-amino-1-naphthol-5,7-disulfonate)- Br_2^{82} , (Dibromo- Br_2^{82} Evans Blue)*. 5,5'-Dibromo-3,3'-dimethyl-4,4'-biphenylenediazonium- Br_2^{82} chloride is coupled with "Chicago Acid" (8-amino-1-naphthol-5,7-disulfonic acid) to obtain the labeled Evans Blue dye. The procedure is the same as for trypan blue with the exception that "Chicago Acid" is more water-soluble than "H-Acid," and the addition of base to effect solution is unnecessary.

B. Notes

1. The color of the solution becomes a dark greenish-purple as the bromine is added. The bromine should be in 10% excess.

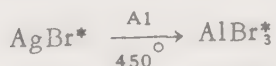
2. The product conforms to a description in the literature¹ and may be used without further purification.

3. Throughout the diazotization an excess of both hydrochloric acid and sodium nitrite should be maintained.

4. In calculating the weight of "H-Acid" to be used and the amount of sodium hydroxide, the purity of the "H-Acid" should be taken into account.

5. The solution turns a deep purple.

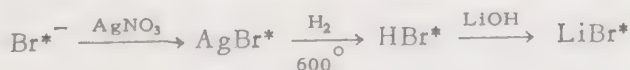
¹W. Schlenk, *Ann.*, 363, 313 (1908).

ALUMINUM BROMIDE-Br⁸⁰₃

F. Fairbrother, *J. Chem. Soc.*, 1941, 293.

Procedure

Aluminum bromide-Br⁸⁰₃ is prepared in a Pyrex glass apparatus comprised of a tube, heated by a furnace and connected to a cold trap and vacuum pump. The active silver bromide is intimately mixed with about 3 times its weight of cleaned aluminum filings (the reaction with aluminum powder is too violent) placed in a glass tube and kept in position by a loose plug of glass wool. This tube is then placed in the furnace tube, and the apparatus is closed and evacuated. At about 400°, the reaction takes place smoothly, and aluminum bromide-Br⁸⁰₃ sublimes to the cool part of the tube. With the aid of a small burner the white product is sublimed into the cold trap.

LITHIUM BROMIDE-Br⁸⁰

C. S. Lu and S. Sugden, *J. Chem. Soc.*, 1939, 1273.

A. Procedure

(a) *Silver Bromide-Br⁸⁰*. Two liters of ethylene dibromide, containing 5% by volume of aniline, is irradiated with neutrons from a radium-beryl-

lithium source for 19 hours (Note 1). The alkyl halide is shaken with 500 ml. of water. The aqueous extract, freed from organic matter by extraction with a little ether, is then treated with silver nitrate. The precipitate of silver bromide- Br^{80} is collected, washed with alcohol and dried rapidly.

(b) *Hydrogen Bromide- Br^{80}* . The dry silver bromide is transferred to a boat and reduced in a current of hydrogen at 600° in a tube-furnace (Note 2).

(c) *Lithium Bromide- Br^{80}* . The resultant hydrogen bromide- Br^{80} is swept with the hydrogen stream into a dilute solution of lithium hydroxide (Note 3).

B. Notes

1. It was demonstrated that the addition of 4-5% of aniline to the organic halide before irradiation increased the percentage of activity extractable with water from 45% to 71%.

2. le Roux and Sugden¹ prepared lithium bromide by the same reactions. They reduced the silver bromide in a silica tube-furnace at $400\text{--}500^\circ$ and showed that 98% of the hydrogen bromide is evolved in 20 minutes.

3. All of the operations were completed in 2-3 hours.

C. Other Preparations

Peri and Daniels² have prepared hydrogen bromide- Br^{82} by mixing 48% hydrobromic acid and irradiated potassium bromide; exchange is rapid. They also prepared hydrogen- H^2 bromide- Br^{82} by heating silver bromide- Br^{82} in contact with hydrogen- H^2 bromide at 400° for 12 hours.

Hydrogen bromide- Br^{82} has been prepared^{3,4} also, by the reduction of silver bromide- Br^{82} with hydrogen.

¹L. J. le Roux and S. Sugden, J. Chem. Soc., 1939, 1279.

²J. B. Peri and F. Daniels, J. Am. Chem. Soc., 72, 424 (1950).

³F. P. W. Winteringham, J. Chem. Soc., 1949, 416.

⁴F. Howarth, Nature, 161, 857 (1948).

TABLE XV, 1
Bromine Exchange with Alkyl Bromides

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
CBr_4	Carbon tetra- bromide	—	AlBr_3^{80}	...	20	15	carbon di- sulfide	89	10% CBr_4 in CS_2 . Organic material isolated by ether extraction.	3
CBr_4	Carbon tetra- bromide	...	Br_2^{82}	170–200	Vapor phase. Rate proportional to conc. of CBr_4 and to square root of conc. of Br_2 . Liquid phase.	4
CBr_4	Carbon tetra- bromide	...	Br_2^{82}	107–160	Liquid phase.	4
CBrCl_3	Bromotrichloro- methane	8.95	Br_2^{82}	0.0282	60	125	...	78.5	Liquid phase. Other data are given.	5
CBrCl_3	Bromotrichloro- methane	0.194	Br_2^{82}	2.74×10^{-3}	30	170	carbon tetra- chloride	86.2	Liquid phase. Other data are given.	5
CBrCl_3	Bromotrichloro- methane	10.6×10^{-3}	Br_2^{82}	3.7×10^{-4}	30	220	...	99.4	Vapor phase.	5
CBrCl_3	Bromotrichloro- methane	...	Br_2^{80}	Exchange catalyzed by intense illumi- nation.	1
CBrCl_3	Bromotrichloro- methane	36.05×10^{-3}	Br_2^{82}	7.3×10^{-3}	601	160.8	...	65	Other data given.	6
CBrCl_3	Bromotrichloro- methane	0.08	Br_2^{82}	3×10^{-3}	75	76	carbon tetra- chloride	3	Thermal exchange in dark.	9
CBrCl_3	Bromotrichloro- methane	0.08	Br_2^{82}	3×10^{-3}	75	76	carbon tetra- chloride	98	Photochemical ex- change with green light.	9

(Continued)

TABLE XV, 1 (Continued)

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
CHBr_3	Tribromomethane	...	AlBr_3^{80}	room	Exchange rapid; equilibrium in less than 1 hour.	2
CHBr_3	Tribromomethane	...	$\text{C}_2\text{H}_5\text{Br}^{80}$	room	No exchange without AlBr_3 catalyst, then rapid.	2
CHBr_3	Tribromomethane	Alkyl halides bombarded with neutrons; a comparative yield study.	7,8
CH_3Br	Bromomethane	...	AlBr_3^{80}	Exchange with bromomethane in gas phase; also in liquid phase.	1
$\text{C}_2\text{H}_2\text{Br}_2$	1,2-Dibromo-ethylene	...	Br_2^{80}	...	240	90	bromobenzene	50	Competitive reaction; 1,2-dibromoethylene and 3-bromopropene present during bombardment with neutrons.	14,15
$\text{C}_2\text{H}_3\text{Br}$	Bromoethylene	...	HBr^{82}	13
$\text{C}_2\text{H}_3\text{Br}$	Bromoethylene	...	AlBr_3^{80}	...	< 2	15	...	97	Product isolated by distillation.	3
$\text{C}_2\text{H}_3\text{Br}_3$	1,1,2-Tribromoethane	...	Br_2^{82}	...	45	140	...	23.5	From 1,2-dibromoethane; substitution and exchange.	13

C_2H_5BrO	Acetyl bromide	...	$AlBr_3^{80}$...	20	15	...	80	...	3
C_2H_5BrO	Acetyl bromide	...	$SnBr_4^{80}$...	25	15	...	62	...	3
$C_2H_4Br_2$	1,2-Dibromoethane	...	HBr^{82}	308-317	...	35	This is % of organically bound activity found in 1,2-dibromoethane; other products present.	13
$C_2H_4Br_2$	1,2-Dibromoethane	...	$AlBr_3^{82}$	Equilibrium in less than 1 hour.	2,10
$C_2H_4Br_2$	1,2-Dibromoethane	...	$AlBr_3^{80}$...	20	15	...	97	Isolated by vacuum distillation.	3
C_2H_5Br	Bromoethane	...	$AlBr_3^{80}$...	10	15	nitrobenzene	2.5	Ratio of nitrobenzene to $AlBr_3$ 2.5 to 1.	3
C_2H_5Br	Bromoethane	...	$SnBr_4^{80}$...	120	15	...	<1	...	3
C_2H_5Br	Bromoethane	...	$AlBr_3^{82}$	Exchange rapid; activation energy not over 10 calories.	2,10
C_2H_5Br	Bromoethane	8×10^{-3}	HBr^{82}	2.8×10^{-4}	18	200	...	81	Increase of surface to volume ratio by adding broken Pyrex tubing gave catalytic effect.	11
C_2H_5Br	Bromoethane	8×10^{-3}	H^2Br^{81}	3.6×10^{-3}	465	258	...	100	Complete to equilibrium. Surface to volume ratio 9 to 1.	11
C_2H_5Br	Bromoethane	...	$AlBr_3^{80}$...	5	15	...	90	Product isolated by vacuum distillation.	3

(Continued)

TABLE XV, 1 (Continued)

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
C_2H_3Br C_3H_3Br	Bromoethane 1-Bromopropene	$AlBr_3^{82}$ Br_2^{80} bromo- benzene 22 A solution of one of the bromopropenes was bombarded.	12 15
C_3H_3Br	1-Bromopropene	1%	Br_2^{80}	540-900	bromo- benzene	18	Solution of 1 ml. of 2-bromopropene in 99 ml. of bromo- benzene was bom- barded with neu- trons. Isomeriza- tion occurred.	14
C_3H_3Br	2-Bromopropene	1%	Br_2^{80}	540-900	bromo- benzene	10	Same as immediately above	14
C_3H_3Br	2-Bromopropene	Br_2^{80}	bromo- benzene	3	A solution of one of the bromopropenes is bombarded.	15
C_3H_3Br	3-Bromopropene	5%	$AlBr_3^{80}$	2	15	carbon di- sulfide	84	Isolated by vacuum distillation.	3
C_3H_3Br	3-Bromopropene	Br_2^{80}	240	90	bromo- benzene	50	Competitive re- action with 1,2- dibromoethylene.	14
C_3H_3Br	3-Bromopropene	1%	Br_2^{80}	bromoethane	25-50	Bromoethane bom- barded with neu- trons, then 3- bromopropene and 1,2-dibromopro- pane added and separated.	15

C_3H_5Br	3-Bromopropene	Br_2^{a0}	bromo- benzene	75	Regardless of which isomeric bromopropene is present during bombardment of C_6H_5Br , isomerization occurs, and 75% of activity is in 3-bromopropene.	15
C_3H_5Br	3-Bromopropene	2.21%	bromo-ethane	48	The solution of 3-bromopropene is bombarded.	14
C_3H_5Br	3-Bromopropene	30	15	2	Isolated by vacuum distillation.	3
$C_3H_5BrO_2$	2-Bromopropionic Acid	0.210	0.090	305	60	1 N sulfuric acid	45.4	The exchange reaction competes with hydrolysis; amount of labeled compound passes through a maximum.	18
$C_3H_5BrO_2$	2-Bromopropionic Acid	0.333	0.333	50	22	acetone	100	At equilibrium, 34% of activity in product; latter isolated by extraction into ether.	20
C_3H_7Br	1-Bromopropane	0.1	0.1	180	100	alcohol	96	Br^- was almost pure Br^{a2} . Exchange is 96% of equilibrium.	17

(Continued)

TABLE XV, 1 (Continued)

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
C_3H_7Br	1-Bromopropane	0.1173	$LiBr^{82}$	0.0349	153	65.9	90% acetone	63.2	Per cent of total activity, Product extracted into benzene.	16
C_3H_7Br	2-Bromopropane	0.093	$LiBr^{82}$	0.0164	945	100	ethylene acetate	68.2	Per cent of total activity, Product extracted into benzene.	21
C_3H_7Br	2-Bromopropane	0.1409	$LiBr^{82}$	0.0275	336	70.3	90% acetone	71.3	This is per cent of total activity. Product extracted into benzene from water.	16
C_4H_9Br	1-Bromobutane	0.1	$LiBr^{82}$	0.0369	450	59.7	90% acetone	100	Equilibrium reached in 7-8 hours.	19
C_4H_9Br	1-Bromobutane	$AlBr_3^{80}$	10	15	97	Isolated by vacuum distillation.	3
C_4H_9Br	1-Bromobutane	$SnBr_4^{80}$	60	15	12	Isolated by vacuum distillation.	3
C_4H_9Br	1-Bromobutane	0.0974	$LiBr^{82}$	0.0222	30	40	dry acetone	50	Per cent of total activity, Product extracted into benzene.	21
C_4H_9Br	1-Bromobutane	0.0922	$LiBr^{82}$	0.0494	30	100.1	ethylene acetate	48.6	Per cent of total activity, Product extracted into benzene.	21

C_4H_9Br	1-Bromobutane	0.045	$LiBr^{a2}$	0.0191	...	26.2	dry acetone	...	Rate constant = 1.07×10^{-3} .	22
C_4H_9Br	1-Bromo-2-methylpropane	0.1509	$LiBr^{a2}$	0.0357	915	70	90% acetone	66.4	Per cent of total activity. Product extracted into benzene.	16
C_4H_9Br	2-Bromo-2-methylpropane	...	$AlBr_3^{a0}$...	5	15	...	100	Product isolated by vacuum distillation.	3
C_4H_9Br	2-Bromo-2-methylpropane	...	$SnBr_4^{a0}$...	150	15	...	92	Product isolated by vacuum distillation.	3
C_4H_9Br	2-Bromo-2-methylpropane	0.105	$LiBr^{a2}$	0.032	13	120	ethylene acetate	45	Per cent of total activity. Product extracted into benzene	21
$C_5H_{11}Br$	1-Bromo-3-methylbutane	...	$AlBr_3^{a2}$	room	10,2
$C_7H_6BrNO_2$	α -Bromo-4-nitrotoluene	0.0106	$LiBr^{a2}$	0.029	20	30	90% acetone	84.5	...	25
$C_7H_6ClNO_2$	α -Chloro-4-nitrotoluene	0.06120	$LiBr^{a2}$	0.04003	...	30	90% acetone	...	$k = 1.35 \pm 0.13 \times 10^{-4}$ 1./mole sec.	25
C_7H_7Br	α -Bromotoluene	...	$AlBr_3^{a0}$	Rapid condensation with evolution of HBr .	3
C_7H_7Br	α -Bromotoluene	...	$SnBr_4^{a0}$...	150	15	...	100	Product isolated by vacuum distillation.	3
C_7H_7Br	α -Bromotoluene	...	$AlBr_3^{a2}$	10,2
$C_7H_{13}Br$	1-Bromo-1-heptene	0.226	$LiBr^{a2}$	0.220	60	158	diethylene glycol	0	...	23

(Continued)

TABLE XV, 1 (Continued)

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
C ₈ H ₇ Br	α-Bromostyrene	0.188	LiBr ⁸²	0.184	60	157	diethylene glycol	13	23
C ₈ H ₇ Br	β-Bromostyrene	0.212	LiBr ⁸²	0.212	60	216	diethylene glycol	4	24
C ₈ H ₉ Br	(1-Bromoethyl)- benzene	0.2	LiBr ⁸²	0.2	21	30.2	dry acetone	Rate constant = $3.49 \pm 0.37 \times 10^{-5}$, Study of Walden inversion.	26
C ₈ H ₁₇ Br	2-Bromoöctane	0.045	LiBr ⁸²	0.015	65.5	Rate constant = 1.77×10^{-3} .	22
C ₂₀ H ₁₃ Br	Bromotriphenyl- ethylene	0.016	LiBr ⁸²	0.176	60	216	diethylene glycol	14.2	24

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TABLE XV, 2
 Bromine Exchange with Aryl Bromides

Formula	Compound	Conc., mol./l.	Isotope source	Conc., mol./l.	Time, min.	Temp., °C.	Solvent	Exchange %	Notes	Ref.
$C_6H_3BrN_2O_4$	1-Bromo-2,4-dinitrobenzene	0.113	$LiBr^{82}$	0.0502	180	130	ethylene acetate	64	Per cent of total activity present appearing in the product.	6
$C_6H_4BrNO_2$	1-Bromo-2-nitrobenzene	0.220	$LiBr^{82}$	0.218	60	158	ethylene glycol	0	No exchange in diethylene glycol, also.	5
C_6H_5Br	Bromobenzene	$AlBr_3^{82}$	30	25	20	1
C_6H_5Br	Bromobenzene	0.215	$LiBr^{82}$	0.216	60	158	diethylene glycol	0	2
C_6H_5Br	Bromobenzene	$Al_2Br_6^{80}$	90	15	3	Degree of exchange at time of separation, taken as ratio of spec. act. in product to that in source.	3
C_6H_5Br	Bromobenzene	$Al_2Br_6^{80}$	90	100	82	11
C_6H_5Br	Bromobenzene	$Al_2Br_6^{80}$	20	room	8
C_6H_5Br	Bromobenzene	0.2	$CuBr_2^{82}$	0.1	30	16	95% ethanol	No exchange.	4
C_6H_5Br	Bromobenzene	0.905 g.	$Al_2Br_6^{82}$	0.086 g.	240	25	Polymerization, very little act.	4
C_6H_5Br	Bromobenzene	0.943 g.	$SbBr_3^{82}$	0.212 g.	240	155	No exchange.	4
$C_{10}H_7Br$	1-Bromonaphthalene	0.416 g.	$CuBr_2^{82}$	0.223 g.	30	200	10 ml. quinoline	No exchange.	4
$C_{10}H_7Br$	1-Bromonaphthalene	0.216	$LiBr^{82}$	0.212	60	242	diethylene glycol	27.7	2
$C_{10}H_7Br$	1-Bromonaphthalene	2.108 g.	$AlBr_3^{82}$	0.148 g.	240	20	Polymerization, some exchange.	4

$C_{10}H_7Br$	1-Bromonaphthalene	1.340 g.	$AlBr_3^{82}$	0.477 g.	60	75	3 ml. benzene	Polymerization, and exchange.	4
$C_{10}H_7Br$	1-Bromonaphthalene	1.250 g.	$AlBr_3^{82}$	0.247 g.	75	75	3 ml. nitrobenzene	No exchange.	4
$C_{10}H_7Br$	1-Bromonaphthalene	1.243 g.	$SbBr_3^{82}$	0.268 g.	240	155	no solvent	No exchange.	4
$C_{10}H_7Br$	1-Bromonaphthalene	$AlBr_3^{82}$	20	room	no solvent	Some exchange.	8
$C_{10}H_7Br$	2-Bromonaphthalene	0.214	$LiBr^{82}$	0.209	60	280	polyethylene glycol	64	2
$C_{10}H_7Br$	1-Bromonaphthalene	0.68	$LiBr^{82}$	0.15	120	260	96% ethanol	~100	Products isolated in ether, washed with silver nitrate.	7
	2-Bromonaphthalene	0.48								
$C_{12}H_9Br$	3-Bromobiphenyl	0.203	$LiBr^{82}$	0.202	80	282	triethylene glycol	67.5	5
$C_{12}H_9Br$	4-Bromobiphenyl	0.205	$LiBr^{82}$	0.205	80	282	triethylene glycol	20	5
$C_{14}H_9Br$	9-Bromoanthracene	0.216	$LiBr^{82}$	0.218	60	280	polyethylene glycol	33	2
$C_{14}H_9Br_2$	9,10-Dibromoanthracene	0.101	$LiBr^{82}$	0.202	60	242	triethylene glycol	6	2

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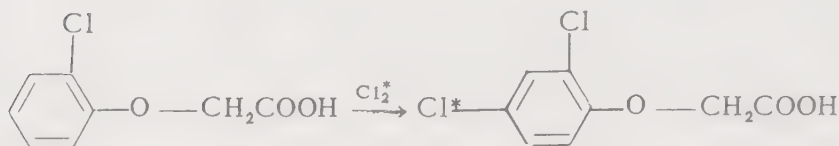
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B. CHLORINE COMPOUNDS

2,4-DICHLOROPHENOXYACETIC-4- Cl^{36} ACID

P. Sørensen, *Anal. Chem.*, 26, 1581 (1954).

A. Procedure (Note 1)

(a) *2,4-Dichlorophenoxyacetic-4- Cl^{36} Acid.* 2-Chlorophenoxyacetic acid, 0.925 g., is dissolved in 10 ml. of acetic acid at 100° and treated with the chlorine- Cl_2^{36} prepared from 1.35 g. of silver chloride- Cl^{36} (Note 2). The solution is then diluted 10-fold with water and heated until clear. The product, which is crystallized by cooling the solution to 0°C ., is collected, washed with water and dried. The yield of 2,4-dichlorophenoxyacetic-4- Cl^{36} acid, m.p. $136\text{--}138^\circ$, is 0.84 g. (Notes 3 and 4).

(b) *2,4,5-Trichlorophenoxyacetic-4- Cl^{36} Acid.* 2,5-Dichlorophenoxyacetic acid, 0.999 g., is chlorinated with the chlorine- Cl_2^{36} from 1.3 g. of silver chloride- Cl^{36} . The yield of product, m.p. $150\text{--}152^\circ$, is 1.15 g.; estimated purity, 90% (Note 5).

(c) *2-Methyl-4-chlorophenoxyacetic- Cl^{36} Acid.* Chlorination of 0.868 g. of 2-methylphenoxyacetic acid is performed with the chlorine- Cl_2^{36} from 1.45 g. of silver chloride- Cl^{36} . The yield of 2-methyl-4-chlorophenoxyacetic- Cl^{36} acid, m.p. $115\text{--}117^\circ$, is 0.85 g.; estimated purity, 95% (Note 6).

(d) *4-Chlorophenoxyacetic- Cl^{36} Acid.* Phenoxyacetic acid, 0.676 g., is chlorinated with the chlorine- Cl_2^{36} from 1.22 g. of silver chloride- Cl^{36} . The yield of 4-chlorophenoxyacetic- Cl^{36} acid, m.p. $154\text{--}157^\circ$, is 0.55 g. (Note 7).

B. Notes

1. The following radioactive compounds were prepared by a method proposed by Haskelberg.¹ These compounds were prepared by Sorensen for use in isotopic dilution analysis procedures for the estimation of the corresponding nonlabeled acids in technical mixtures.

2. A less pure product was obtained when 4-chlorophenoxyacetic acid was used as the starting material.

3. The active preparation was diluted with 4.50 g. of pure 2,4-dichlorophenoxyacetic acid, and this mixture was recrystallized twice from 40-ml. portions of benzene; yield 4.8 g.; m.p. $140\text{--}140.5^\circ$.

4. The by-product hydrogen chloride- Cl^{36} , together with small amounts of unreacted chlorine- Cl_2^{36} , was absorbed in water containing 1% sodium hydrogen sulfite and precipitated again as silver chloride- Cl^{36} . The filtrate from the crude 2,4-dichlorophenoxyacetic-4- Cl^{36} acid was reduced with Raney nickel-aluminum alloy,² and the liberated chlorine- Cl^{36} was precipitated as silver chloride- Cl^{36} also. The yield of crude product was about 80% with respect to the amount of chlorine consumed.

5. An analytical sample melted at 156.0–157.0°.

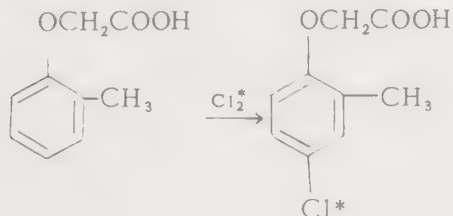
6. A pure sample of 2-methyl-4-chlorophenoxyacetic acid melted at 119.5–120°.

7. Pure 4-chlorophenoxyacetic acid, 3.40 g., was added to the active material and the mixture was recrystallized twice from 100-ml. portions of benzene; yield 3.75 g., m.p. 158–159°. An analytical sample melted at 158.0–159.0°.

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(4-CHLORO-*o*-TOLYLOXY)ACETIC- Cl^{36} ACID



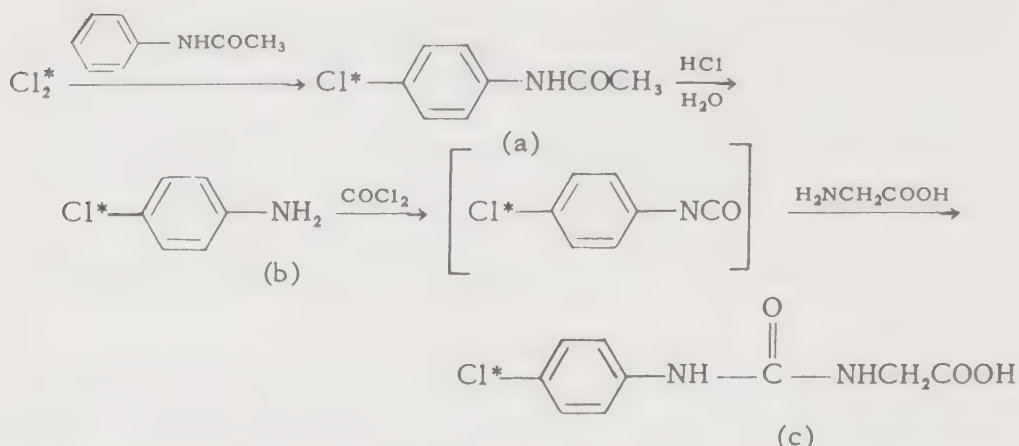
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A. Procedure

o-Tolyloxyacetic acid, 1.03 g., is dissolved in 10 g. of acetic acid at 100° and reacted with the chlorine- Cl_2^{36} from 1.947 g. of silver chloride- Cl^{36} . The acetic acid solution is diluted with 15 volumes of water, again heated to obtain a clear solution and cooled to crystallize the product. The crystalline (4-chloro-*o*-tolyloxy)acetic- Cl^{36} acid is collected and dried, m.p. 118.5–119°; yield, 0.964 g. (Note 1).

B. Notes

1. The purity was estimated to be 95%. Chlorine-containing by-products, e.g. HCl, were converted to silver chloride, and the isotopic yield was estimated to be 80%.

5-(4-CHLOROPHENYL)HYDANTOIC- Cl^{36} ACID

H. H. Woeber, J. Am. Chem. Soc., 74, 1354 (1952).

A. Procedure

(a) *4'-Chloroacetanilide- Cl^{36}* . The chlorine- Cl_2^{36} (22.5 mg.) from 1.03 *N* hydrochloric- Cl^{36} acid (Note 1) is swept through a gas dispersion tube into a solution of 516 mg. of acetanilide in 30 ml. of water, with a slow stream of air. The effluent air is passed through 5 ml. of 0.1 *N* sodium hydroxide. After the reaction mixture cools, precipitate adhering to the gas dispersion tube is washed into the mixture with water, and the sodium hydroxide solution from the trap is added. After the mixture is cooled in ice for 2 hours, mother liquor is removed with a filter stick, the precipitate is washed with cold water and heated to boiling, and the hot solution is filtered through a fritted glass funnel. The reaction tube and filter are washed with 5 ml. of hot water. Upon cooling, 23.8 mg. (28.6%) of 4'-chloroacetanilide- Cl^{36} , m.p. 178° , crystallizes from the solution.

(b) *4'-Chloroaniline- Cl^{36} Hydrochloride*. The 4'-chloroacetanilide- Cl^{36} is heated under reflux with 0.5 ml. of concentrated hydrochloric acid for 1 hour. The excess acid is evaporated with a stream of dry air while the mixture is warmed at $50\text{--}60^\circ$.

(c) *5-(4-Chlorophenyl)hydantoic- Cl^{36} Acid*. One ml. of dry, pure dioxane is added to the 4'-chloroaniline- Cl^{36} hydrochloride. Phosgene (Note 3) is bubbled through the solution for 40–45 minutes; then the solution is heated until dioxane begins to distill. The reaction tube is connected to a soda lime tube, immersed in an ice-bath, the excess phosgene is removed under reduced pressure, and a solution of 30 mg. of glycine in 1 ml. of water and an equivalent amount of sodium hydroxide are added. The solution is stirred vigorously for 10 minutes, cooled in an ice-bath for 1 hour and filtered. Upon addition of 1 equivalent of hydrochloric acid (Note 4), a precipitate forms immediately. After the mixture is

cooled in an ice-bath, the precipitate is collected and washed with cold water. The product is purified by repeating this procedure using 1 ml. of 0.1 *N* sodium hydroxide. The yield of 5-(4-chlorophenyl)hydantoic-Cl³⁶ acid is 17.6 mg. (15.6%); m.p. 189–191° (dec.) (Notes 5 and 6).

B. Notes

1. Woeber used the hydrogen peroxide method of oxidation; a diagram of his simple, compact apparatus is given in the original literature.

2. Adhering solids are washed from the filter stick with acetone, which is then evaporated with a stream of dry air.

3. The phosgene is purified by passing it through peanut oil and then concentrated sulfuric acid.

4. Equivalent to the sodium hydroxide just added.

5. Analyses and melting points were obtained from nonradioactive materials prepared by the same procedure.

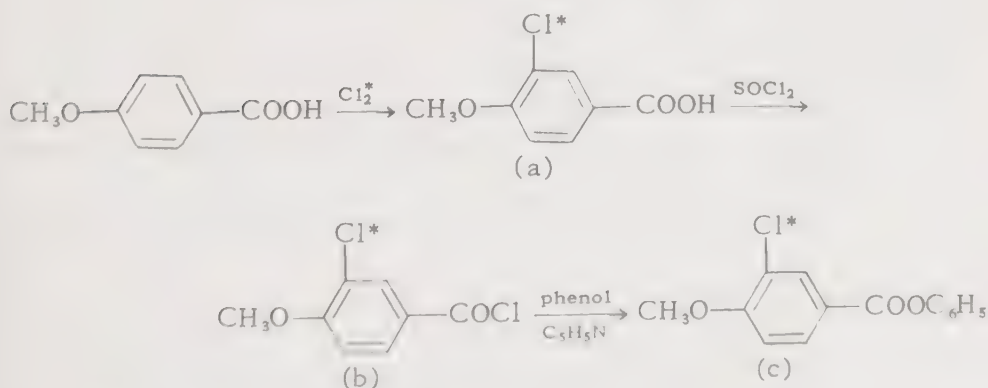
6. Preliminary experiments indicated that 60–80% of the unused chlorine-Cl³⁶ in mother liquors and washings can be converted to sodium chloride-Cl³⁶ for reuse.

C. Other Preparations

4'-Chloroacetanilide-Cl³⁶, m.p. 175–177°, has been obtained¹ in a yield of 0.64 g. (37.7%) by chlorination of 1.35 g. (0.01 mole) of acetanilide dissolved in 10 ml. of glacial acetic acid.

¹J. M. Gryder, M. F. Argus, M. P. Newell and F. E. Ray, J. Am. Pharm. Assoc., 43, 667 (1954).

PHENYL 3-CHLORO-*p*-ANISATE-Cl³⁶



P. Sørensen, Anal. Chem., 27, 388 (1955).

A. Procedure (Note 1)

(a) *3-Chloro-p-anisic-Cl³⁶ Acid*. Chlorine-Cl³⁶, produced from 11.08 g. of silver chloride-Cl³⁶, is absorbed in 120 ml. of 1 *N* sodium hydroxide contained in a flask equipped with a dropping funnel, and 3.90 g. of *p*-anisic acid is added. The flask is closed and evacuated to a pressure of about 200 mm., and 130 ml. of 1 *N* nitric acid is added from the dropping funnel with shaking of the mixture. Then 10 ml. of 0.5% solution of sodium hydrosulfite is added to reduce any unreacted chlorine-Cl³⁶. The precipitate of 3-chloro-*p*-anisic-Cl³⁶ acid is collected, dried and crystallized twice from 75-ml. portions of toluene. The yield of product, m.p. 216–217°, is 3.22 g. (Note 2).

(b) *3-Chloro-p-anisoyl-Cl³⁶ Chloride*. 3-Chloro-*p*-anisic-Cl³⁶ acid is refluxed with 4 to 6 times the theoretical amount of thionyl chloride for 15 minutes after a clear solution results. Excess thionyl chloride is then removed under reduced pressure.

(c) *Phenyl 3-Chloro-p-anisate-Cl³⁶*. The residue of crude 3-chloro-*p*-anisoyl-Cl³⁶ chloride is refluxed for 30 minutes with a 100% excess of phenol dissolved in pyridine. The reaction mixture is then poured into water, and the crude product is crystallized and recrystallized from an organic solvent (Note 3).

A number of derivatives of 3-chloro-*p*-anisic-Cl³⁶ acid, prepared in this manner, are listed in Table XV, 3. The yields of these esters and amides,

TABLE XV, 3

Derivatives of 3-Chloro-*p*-Anisic-Cl³⁶ Acid

Compound	M.p., °C.
Phenyl 3-chloro- <i>p</i> -anisate-Cl ³⁶	141.7–142.0
<i>o</i> -Phenylene bis(3-chloro- <i>p</i> -anisate-Cl ³⁶)	174.4–174.9
Methyl 3-chloro- <i>p</i> -anisate-Cl ³⁶	94.0–94.3
Ethylene bis(3-chloro- <i>p</i> -anisate-Cl ³⁶)	186.5–186.7
3-Chloro- <i>p</i> -anisilide-Cl ³⁶	169.2–169.5
<i>N,N'</i> -Ethylenebis(3-chloro- <i>p</i> -anisamide-Cl ³⁶)	243.7–244.1

based on 3-chloro-*p*-anisic-Cl³⁶ acid consumed, are 60 to 90%. The melting points given in the table are of pure nonisotopic compounds; the melting points of the corresponding isotopic compounds differed not more than a few tenths of a degree.

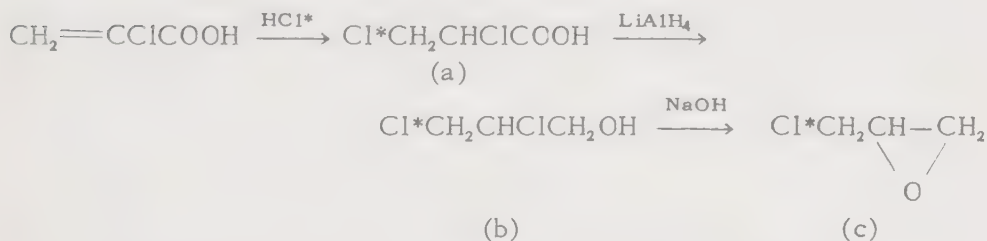
B. Notes

1. These compounds were prepared during the development of an isotopic dilution method of determining hydroxy and amino compounds.

2. Chlorine-36 was recovered from chlorine-containing fractions as silver chloride- Cl^{36} . The yield of 3-chloro-*p*-anisic- Cl^{36} acid based on chlorine- Cl_2^{36} consumed was about 75%.

3. After the mother liquors from the crystallizations were saponified, some 3-chloro-*p*-anisic- Cl^{36} acid could be recovered.

EPICHLOROHYDRIN- Cl^{36}



P. B. D. de la Mare and J. G. Pritchard, J. Chem. Soc., 1954, 1644.

A. Procedure

(a) *2,3-Dichloropropionic-3- Cl^{36} Acid* (Note 1). A solution of 9.8 g. of dry hydrogen chloride- Cl^{36} in 100 ml. of acetic acid is added to 22.4 g. of 2-chloroacrylic acid (Note 2). After the reaction proceeds for 64 hours at 45° , the product is fractionated to obtain 15 g. of the acid, b.p. $114-118^\circ$ (14.5 mm.), which solidifies. The product is recrystallized from petroleum ether, m.p. $51.5-53^\circ$.

(b) *2,3-Dichloro-1-propanol-3- Cl^{36}* . To a solution of 17.5 g. of 2,3-dichloropropionic-3- Cl^{36} acid in 70 ml. of dry ether is added 4.5 g. of lithium aluminum hydride in 100 ml. of dry ether. The mixture is refluxed for 15 minutes and then is treated with 250 ml. of 2 *N* sulfuric acid. The ether solution is dried, first with potassium carbonate and then with sodium sulfate. Removal of ether and fractionation of the product give 7 g. of 2,3-dichloro-1-propanol-3- Cl^{36} , b.p. $78-79^\circ$ (13 mm.), n_D^{25} 1.4822 (Note 3).

(c) *Epichlorohydrin- Cl^{36}* . 2,3-Dichloro-1-propanol-3- Cl^{36} , 12.65 g., is treated with 99 ml. of 1 *N* sodium hydroxide at 0° for 18 hours. The product is extracted with ether; the extract is dried over sodium sulfate and fractionated. The yield of epichlorohydrin- Cl^{36} is 2 ml.; b.p. $117-118$ (760 mm.), n_D^{25} 1.4358 (Note 4).

B. Notes

1. Preparation of this acid is according to the method of Werigo and Melikov.¹

2. Preparation of 2-chloroacrylic acid is described by de la Mare and Pritchard.

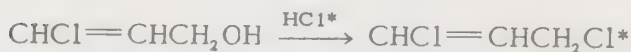
3. It has been shown by Lennart Smith² that the isomeric dichlorohydrins, 2,3-dichloro-1-propanol and 1,3-dichloro-2-propanol, can be distinguished by their rates of reaction with alkali. de la Mare and Pritchard prepared pure 2,3-dichloro-1-propanol; b.p. 182° (760 mm.), 71.7-72.2° (10 mm.), n_D^{25} 1.4822. Reaction of this material with alkali (both reactants 0.05 N) had $k = 0.0100$ l. mole⁻¹ min.⁻¹ at 0.0° and 0.321 l. mole⁻¹ min.⁻¹ at 25.0°. Absence of both a systematic trend in the rate-coefficients and any appreciable rapid initial reaction showed the absence of the isomeric 1,3-dichloro-2-propanol. The reaction of the isotopic compound with sodium hydroxide (both reactants 0.05 N) had $k = 0.321$ l. mole⁻¹ min.⁻¹ at 25°.

4. On a preparative scale in which the reaction is allowed to go to completion, the yield of epichlorohydrin is only about 55%. A considerable amount of complex high-boiling material, b.p. 90-100° (18 mm.), is also obtained. In a separate experiment with 2,3-dichloro-1-propanol-3-Cl³⁶ in which the reaction was allowed to go to completion, it was shown that some of the sodium chloride formed in the reaction came from the 3-carbon atom.

¹Werigo and Melikov, Ber., 10, 1499 (1877).

²L. Smith, Z. physik. Chem. (Leipzig), 92, 740 (1917).

cis- AND *trans*-1,3-DICHLOROPROPENE-3-Cl³⁶



L. F. Hatch, L. O. Morgan and V. L. Tweedie, J. Am. Chem. Soc., 74, 1826 (1952).

A. Procedure

(a) *cis*-1,3-Dichloropropene-3-Cl³⁶. In an all-glass apparatus (Note 1), 8.68 g. (0.24 mole) of hydrogen chloride-Cl³⁶ is passed slowly, during 11 hours, into a stirred mixture of 31.7 g. (0.34 mole) of *cis*-3-chloro-2-propen-1-ol and 0.40 g. (0.0047 mole) of zinc oxide at 70 ± 5° (Note 2). The reaction mixture is poured into 25 ml. of water and extracted several times with small portions of ether (3-5 ml.). The extract, after drying over sodium sulfate, is fractionated in a 15-inch Vigreux column under reduced pressure. Based on the hydrogen chloride, the conversion is 73% and the yield 71% of *cis*-1,3-dichloropropene-3-Cl³⁶, n_D^{25} 1.4651 (Note 3).

(b) *trans*-1,3-Dichloropropene-3-Cl³⁶. The *trans*-isomer is prepared from *trans*-3-chloro-2-propen-1-ol in the same manner as the *cis*-isomer above. Conversion of hydrogen chloride is 76%, and the yield is 68% of *trans*-1,3-dichloropropene-3-Cl³⁶, n_D^{25} 1.4710 (Note 4).

B. Notes

1. The apparatus used in the preparation of the 1,3-dichloropropenes from the corresponding alcohols was constructed entirely of glass and consisted essentially of a hydrogen chloride generator, a 100-ml. reaction vessel equipped with a stirrer, thermometer, gas inlet tube, condenser and a trap for recovery of unreacted hydrogen chloride- Cl^{36} .

2. Unreacted hydrogen chloride- Cl^{36} is swept, with a stream of nitrogen, into a water-trap for recovery.

3. The literature¹ gives n_D^{25} 1.4652.

4. The literature¹ gives n_D^{25} 1.4712.

¹L. F. Hatch and R. F. Perry, Jr., J. Am. Chem. Soc., 71, 3262 (1949).

2-CHLORO-2-METHYLPROPANE- Cl^{36}

(*t*-Butyl Chloride- Cl^{36})



W. Koskoski, H. Thomas and R. D. Fowler, J. Am. Chem. Soc., 63, 2451 (1941).

A. Procedure

The exchange (Note 1) is carried out in a single reaction vessel. A solution of lithium chloride- Cl^{36} (Note 2) in dry formic acid is placed in the flask, and to this is added the *t*-butyl chloride (Note 3). At the end of the reaction period, the mixture is added to a suitable quantity of cold ether and washed twice with an equal volume of water to remove the lithium chloride. To obtain the *t*-butyl chloride (b.p. 49.7° at 742.4 mm.), the ether layer is washed successively with sodium bicarbonate solution and water, and is then dried and fractionated.

A kinetic study indicated that the exchange at 19° was 90.4% complete in 2 hours.

B. Notes

1. Bateman and Hughes¹ have shown that when *t*-butyl chloride is placed in dry formic acid an equilibrium is established rapidly between the *t*-butyl chloride, chloride ions and isobutylene. Therefore, upon adding isotopic chloride ion to the system, exchange should progress through the equilibrium.

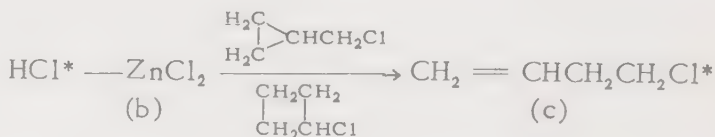
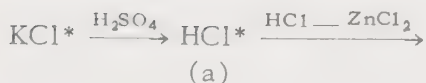
2. The radioactive chlorine was prepared by bombarding chloroform with neutrons. After irradiation, the active chlorine is removed from the chloroform by shaking it with water containing a little lithium chloride. The aqueous solution is then evaporated to dryness, the vessel is swept

out with dry nitrogen, and the lithium chloride is taken up in dry formic acid.

3. For the kinetic studies Koskoski, *et al.*, used single reaction vessels and also Y-shaped sealed vessels. In the latter, the *t*-butyl chloride was placed in one side-arm and the lithium chloride solution in the other; then the vessels were sealed and placed in a bath at 15°. After thermal equilibrium was attained, the vessels were inverted and shaken. At selected times, the vessels were opened and the contents were poured into 25-ml. quantities of cold ether. The lithium chloride was extracted into water, and chloride ion precipitated as silver chloride which was collected, washed with water and acetone, dried and assayed for activity.

¹L. C. Bateman and E. D. Hughes, J. Chem. Soc., 1937, 1187.

4-CHLORO-1-BUTENE-Cl³⁸



J. D. Roberts and R. H. Mazur, J. Am. Chem. Soc., 73, 2509 (1951).

A. Procedure

(a) *Hydrogen Chloride-Cl³⁸*. One gram of potassium chloride-Cl³⁸ (Note 1), in an all-glass distillation apparatus, is treated with an excess of concentrated sulfuric acid. The resulting hydrogen chloride is swept, with a stream of carbon dioxide, into a receiver containing 1 ml. of water.

(b) *Zinc Chloride-Hydrochloric-Cl³⁸ Acid, (Lucas Reagent¹)*. The hydrochloric-Cl³⁸ acid is added to 30 g. of ordinary Lucas reagent.

(c) *4-Chloro-1-butene-Cl³⁸*. A simultaneous exchange reaction and isomerization is carried out by stirring 2.11 g. of a mixture of (chloromethyl)cyclopropane (about 65%) and cyclobutyl chloride (about 35%) with 6.36 g. of the Lucas reagent for 1 hour at room temperature. Then 20 ml. each of water and methylene chloride are added, the mixture shaken, and the layers are separated. The aqueous and organic layers are washed with methylene chloride and water respectively, and the combined methylene chloride solution is dried over sodium sulfate. Radioactivity assay of aliquots of this solution indicate approximately 100% exchange of the organic chlorine.

In a similar experiment with 4-chloro-1-butene and the active Lucas reagent, only 13% exchange of chlorine took place. Therefore, it is concluded that about 80% of the mixture is isomerized to the 4-chloro-1-butene-Cl³⁶ (Note 2).

B. Notes

1. Potassium chloride, 1 g., was bombarded with deuterons (cyclotron) for 10 minutes. The target material was allowed to stand for 30 minutes and was then transferred to an all-glass distillation apparatus.

2. In a similar experiment with inactive materials, a 46% yield of 4-chloro-1-butene, b.p. 75-76°, n_D^{25} 1.4185, was isolated by distillation.

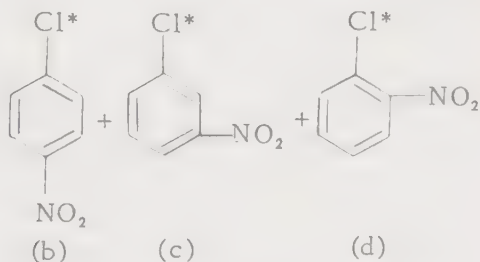
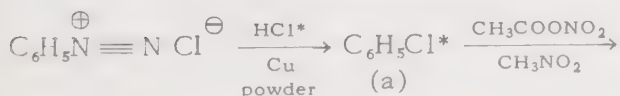
C. Other Preparations

Hydrogen chloride-Cl³⁶ has been prepared² by dropping concentrated sulfuric acid on potassium chloride-Cl³⁶.

¹H. J. Lucas, J. Am. Chem. Soc., 52, 802 (1930).

²L. F. Hatch, L. O. Morgan and V. L. Tweedie, J. Am. Chem. Soc., 74, 1826 (1952).

1-CHLORO-2-NITROBENZENE-Cl³⁶



J. D. Roberts, J. K. Sanford, F. L. J. Sixma, H. Cerfontain and R. Zagt, J. Am. Chem. Soc., 76, 4524 (1954).

A. Procedure

(a) *Chlorobenzene-Cl³⁶*. To a thick slurry of 42.19 g. (0.30 mole) of benzenediazonium chloride (Note 1) in 250 ml. of anhydrous dioxane is added 4.7 ml. of 1.03 *N* hydrochloric-Cl³⁶ acid followed by 115 ml. of anhydrous dioxane (Note 2). With stirring, 30 minutes is allowed for equilibration of various species of chloride ion, and 3 g. of dry copper powder is then added gradually during 10 minutes. Following a brief

induction period, nitrogen evolution becomes rapid, and within 15 minutes, 6 l. (91%) of gas is evolved. The rate of reaction is controlled by slow addition of the catalyst, rapid and efficient stirring, and external cooling, such that the internal temperature does not exceed 29° .

The light-amber liquid is made alkaline and steam-distilled. The dioxane, salted out of the first part of the steam distillate with calcium chloride, is added to the ether used to continuously extract the aqueous phase. Ether and dioxane are removed from the combined extract through a 100-cm. glass, helix-packed column. The yield of chlorobenzene- Cl^{36} , n_{D}^{25} 1.5163–1.5171, is 3.10 g. (9.5%), after extensive purification by distillation through a Podbielniak vacuum-jacketed concentric-tube column (Note 3).

Nitration of Chlorobenzene- Cl^{36} . Chlorobenzene- Cl^{36} , 2.867 g. (0.0255 mole), dissolved in 11.5 ml. of nitromethane, is nitrated at 25° with 0.038 mole of acetyl nitrate (Note 4), according to the procedure of Bird and Ingold.¹ After 54 hours, the reaction mixture is diluted to 50 ml. with ether.

(b) *1-Chloro-4-nitrobenzene- Cl^{36} .* To 40 ml. (Note 5) of the above ether solution is added a mixture of 10.0625 g. of 1-chloro-3-nitrobenzene, 10.0056 g. of 1-chloro-4-nitrobenzene, 10.0440 g. of chlorobenzene, sodium chloride and chlorine (11.5 meq.) in 25 ml. of water. Ice is added to hydrolyze the acetyl nitrate. The ether phase is washed with 20 ml. of 5% sodium bisulfite solution (Note 6), dried over calcium chloride, concentrated and cooled to -80° . The crystalline product, 6.2 g., is separated, dissolved in benzene and chromatographed on a 40×100 -mm. column of alumina. The adsorbed material, visible as a purple band under ultraviolet light, is eluted with pentane in two portions. The mother liquor is also chromatographed.

The 1-chloro-4-nitrobenzene- Cl^{36} fraction, identified by its infrared spectrum, is further purified by chromatography after it is mixed with 1 g. of 1-chloro-2-nitrobenzene. The first fraction is 1-chloro-4-nitrobenzene- Cl^{36} , m.p. 83.1 – 83.6° , which is again scavenged with 1 g. of *o*-isomer and 2 ml. of chlorobenzene to obtain 1.14 g. of products, m.p. 83.4 – 84.2° .

(c) *1-Chloro-3-nitrobenzene- Cl^{36} .* The second fractions of the original chromatographic separations contain most of the chlorobenzene- Cl^{36} and the 1-chloro-3-nitrobenzene- Cl^{36} . The combined fractions are refluxed for 2 hours with 2 g. each of 1-chloro-2-nitrobenzene and 1-chloro-4-nitrobenzene and 50 ml. of piperidine. The piperidine hydrochloride is removed and this treatment is repeated 4 times; finally, the reaction mixture is acidified and steam-distilled. The distillate is extracted with ether, and the extracts are washed with sulfuric acid, sodium hydroxide and water, and dried over calcium chloride. After removal of ether,

the mixture (Note 7) is chromatographed on alumina with pentane. The first fraction is a mixture of chlorobenzene and benzene. The following fractions yield 1.9 g. of 1-chloro-3-nitrobenzene-Cl³⁶, m.p. 45.3–45.8°, after recrystallization from pentane.

(d) *1-Chloro-2-nitrobenzene-Cl³⁶*. The crude *o*-isomer recovered from the chromatographic separations is mixed with inactive *p*- (2 g.) and *m*-isomers (0.3 g.) and chlorobenzene (2 ml.). After chromatographic separation, the recovered 1-chloro-2-nitrobenzene-Cl³⁶ melts at 32.5–33.2°.

B. Notes

1. Anhydrous benzenediazonium chloride was prepared by the method of Smith and Waring.²

2. The reaction was carried out in a 1 l. 3-necked flask equipped with a thermometer well, an addition tube for solids, a gas outlet, and a high-speed stirrer. The gas outlet was connected through two Dry Ice-traps to a wet-test gas meter. The reaction flask was cooled in a calcium chloride-ice bath.

3. The product appeared spectroscopically pure except for two quite small unidentified infrared absorption bands at 769 and 795 cm⁻¹.

4. Acetyl nitrate was prepared from equivalent amounts of anhydrous nitric acid and acetic anhydride at 0°.

5. A 3.00-ml. fraction (6%) was used for determining the total activity of the reaction mixture.

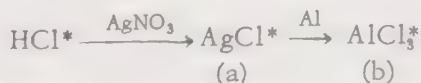
6. The combined aqueous layers were washed with ether, acidified, boiled with charcoal and used for the determination of chloride-Cl³⁶ activity.

7. Infrared analysis indicated some unidentified material and about equal amounts of chlorobenzene and the *m*-isomer.

¹M. L. Bird and C. K. Ingold, J. Chem. Soc., 1938, 918.

²W. Smith and C. E. Waring, J. Am. Chem. Soc., 64, 469 (1942).

ALUMINUM CHLORIDE-Cl³⁶



C. H. Wallace and J. E. Willard, J. Am. Chem. Soc., 72, 5275 (1950).

A. Procedure

(a) *Silver Chloride-Cl³⁶*. Silver chloride-Cl³⁶ is prepared by the addition of silver nitrate to a solution of hydrochloric-Cl³⁶ acid. To insure dryness, the silver chloride-Cl³⁶ is fused under vacuum (Note 1).

TABLE XV, 4
 Chlorine Exchange Reactions

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time	Temp, °C.	Solvent	% Ex- change	Notes	Ref.
CCl ₄	Carbon tetra- chloride	~10.3	AlCl ₃ ³⁶	1 × 10 ⁻³	26 hr.	51	CCl ₄	100	Very small amounts of water (10 ⁻⁵ mole/l.) have no ef- fect, but larger amounts diminish the rate.	2
CCl ₄	Carbon tetra- chloride	AlCl ₃ ³⁶	25 min.	25	9.8	Organic compound in vapor phase.	3
CClF ₃	Chlorotrifluoro- methane	HCl ³⁷	2 hrs.	405	No exchange.	1
CHCl ₃	Chloroform	12.5	AlCl ₃ ³⁶	1 × 10 ⁻³	13 min.	55-90	CHCl ₃	100	Product was fractionated with carbon tetrachloride added as carrier. Up to 20% of activity present as carbon tetrachloride.	2
CHCl ₃	Chloroform	Cl ₂ ³⁶	7 hrs.	97	water	5
CHCl ₃	Chloroform	AlCl ₃ ³⁶	5 min.	25	2.9	Organic compound in vapor phase.	3
CHCl ₃	Chloroform	HCl ³⁶	70-146 hrs.	20-26	water	5
CHClF ₂	Chlorodifluoro- methane	625 mm.	HCl ³⁷	125 mm.	2 hrs.	420	39	k _{1/2,1/2} = 2.6 ± 0.5 × 10 ⁻⁵ sec. ⁻¹ .	1
CH ₂ ClF	Chlorofluoro- methane	625 mm.	HCl ³⁷	125 mm.	2 hrs.	480	66	k _{1,0} = 2.5 ± 0.05 × 10 ⁻⁵ sec. ⁻¹ .	1
CH ₃ Cl	Chloromethane	125 mm.	HCl ³⁷	125 mm.	1.5 hrs.	465	100	k _{1,0} = 16.7 ± 0.3 × 10 ⁻⁵ sec. ⁻¹ .	1
C ₂ H ₅ Cl	Chloroethane	AlCl ₃ ³⁶	5 min.	25	3.8	Organic compound in vapor phase.	3

C_3H_7Cl	1-Chloropropane	9.5	$AlCl_3^{36}$	0.1	15 hrs.	25	...	69.4	Organic compound in vapor phase.	3
C_4H_9Cl	1-Chlorobutane	9.5	$AlCl_3^{36}$	0.1	1-chloro-butane	95	Some moisture present initially. Distillation range of product, 76-77°.	2
C_4H_9Cl	2-Chlorobutane	9.4	$AlCl_3^{36}$	0.1	2-chloro-butane	90	Some moisture present initially. Distillation range of product, 68-69°.	2
C_4H_9Cl	2-Chloro-2-methylpropane	9.1	$AlCl_3^{36}$	0.1	2-chloro-2-methylpropane	90	Some moisture present initially. Distillation range of product, 50-52°.	2
C_4H_9Cl	2-Chloro-2-methylpropane	...	$SnCl_4^{36}$...	5 min.	25	2-chloro-2-methylpropane	70	...	7
C_5H_9Cl	3-Chloro-3-methyl-1-butene	0.37	$LiCl^{36}$	0.675	4.8 min.	25	75% ethanol	37-63	Product was mixture of 3-chloro-3-methyl-1-butene and 1-chloro-3-methyl-2-butene.	8
$C_5H_{11}Cl$	1-Chloropentane	~8.2	$AlCl_3^{36}$	0.1	1-chloropentane	70	Some moisture present initially. Distillation range of product, 100-105°.	2
$C_5H_{11}Cl$	1-Chloropentane	...	$AlCl_3^{36}$...	5 min.	25	...	4.9	Organic compound in vapor phase.	3
$C_7H_6BrNO_2$	α -Bromo-4-nitrotoluene	0.05289	$LiCl^{36}$	0.04169	...	30	90% acetone	...	$k = 21.4 \pm 1.7 \times 10^{-4}$ l./mole sec.	6
$C_7H_6ClNO_2$	α -Chloro-4-nitrotoluene	0.05771	$LiCl^{36}$	0.01146	214 min.	30	90% acetone	1.6	$k = 0.187 \times 10^{-4}$ l./mole sec.	6
C_7H_7Cl	α -Chlorotoluene	...	$AlCl_3^{36}$...	5 min.	50	...	0.3	Organic compound in vapor phase.	3

(Continued)

TABLE XV, 4 (Continued)

Formula	Compound	Conc. ml./l.	Isotope source	Conc. mol./l.	Time	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
C ₉ H ₁₉ Cl	Camphene · HCl	0.158	HCl ³⁸	0.075	10	20	CHCl ₃ , C ₆ H ₅ NO ₂	62	This is 100% of calculated equilibration value. There is 5% isomerization to iso- bornyl chloride in this time.	4
C ₉ H ₁₉ Cl	Camphene · HCl	0.158	HCl ³⁸	0.075	20	0	CHCl ₃	20	Per cent of total chlorine isotope.	4
C ₉ H ₁₉ Cl	Camphene · HCl	0.149	H ² Cl ³⁸	0.0833	20	0	CHCl ₃	20	Per cent of total chlorine iso- tope. Exchange of deuter- ium for hydrogen parallels rate of chlorine exchange.	4

¹J. E. Boggs and L. O. Brockway, J. Am. Chem. Soc., 77, 3444 (1955).²C. H. Wallace and J. E. Willard, *ibid.*, 72, 5275 (1950).³M. Blair and J. E. Willard, *ibid.*, 73, 442 (1951).⁴T. P. Nevell, E. de Salas and C. L. Wilson, J. Chem. Soc., 1939, 1188.⁵J. Horiuchi and K. Tanabe, Proc. Japan Acad., 27, 404 (1951); through Chem. Abstracts, 46, 7417 (1952); *ibid.*, 28, 130 (1952); through Chem. Abstracts, 46, 7902 (1952).⁶T. A. Bither, J. M. Sturtevant and H. C. Thomas, J. Am. Chem. Soc., 67, 1562 (1945).⁷R. A. Howald and J. E. Willard, *ibid.*, 77, 2048 (1955).⁸P. B. D. de la Mare and C. A. Vernon, J. Chem. Soc., 1954, 2504.

(b) *Aluminum Chloride-Cl₃³⁶*. When a good vacuum is re-established in the system, after removal of water vapor, an excess of 30-mesh aluminum (Note 2) is then added from a side-arm by jarring or tipping the apparatus. The mixture is heated until, at about 450°, rapid formation of aluminum chloride-Cl₂³⁶ occurs. The product sublimes into an adjacent flask cooled with liquid nitrogen.

B. Notes

1. When moisture is occluded in the silver chloride, its presence can be determined after the synthesis by freezing out and counting the equivalent amount of hydrogen chloride which is formed by hydrolysis of aluminum chloride. Such tests showed that silver chloride dried at 160° for 4 hours contained about 1% moisture. The silver chloride fused in the vacuum system was completely dry.

2. The aluminum metal, cleaned by immersion in aqueous hydrochloric acid, is rinsed with water and dried.

C. Other Preparations

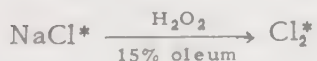
Silver chloride-Cl³⁶ has been obtained¹ from sodium chloride-Cl³⁶ and silver nitrate.

Blau and Willard² also prepared aluminum chloride-Cl₃³⁶ by heating silver chloride-Cl³⁶ and aluminum metal and subliming the product under vacuum.

¹P. Sørensen, *Acta Chem. Scand.*, 5, 630 (1951).

²M. Blau and J. E. Willard, *J. Am. Chem. Soc.*, 73, 442 (1951).

CHLORINE-Cl₂³⁶



H. H. Woeber, *J. Am. Chem. Soc.*, 74, 1354 (1952).

A. Procedure

To 1 mmole of sodium chloride-Cl³⁶, dissolved in 1 ml. of 30% hydrogen peroxide and cooled in ice, is added 3 ml. of cold, fuming sulfuric acid (15% SO₃). The evolved chlorine-Cl₂³⁶, about 35 mg. (95-100%), is collected in a suitable cold trap, cooled with Dry Ice or liquid nitrogen, or passed directly into a reaction mixture.

B. Other Preparations

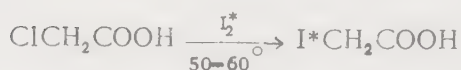
Sörensen¹ prepared chlorine- Cl^{36} in 95% yield by the oxidation of silver chloride- Cl^{36} with potassium dichromate in sulfuric acid. Townes and Aamodt² oxidized sodium chloride- Cl^{36} with manganese dioxide in concentrated sulfuric acid, and Gryder, *et al.*³ oxidized hydrogen chloride- Cl^{36} with permanganate in concentrated sulfuric acid.

¹P. Sörensen, *Acta Chem. Scand.*, 5, 630 (1951); *Anal. Chem.*, 26, 1581 (1954)

²C. H. Townes and L. C. Aamodt, *Phys. Rev.*, 76, 691 (1949).

³J. M. Gryder, M. F. Argus, M. P. Newell and F. E. Ray, *J. Am. Pharm. Assoc., Sci. Ed.*, 43, 667 (1954).

C. IODINE COMPOUNDS

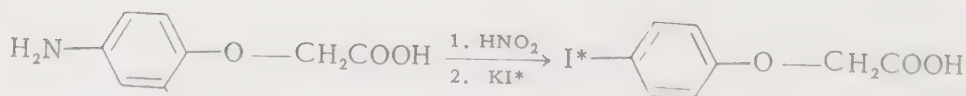
IODOACETIC- I^{31} ACID

P. R. Thomassen and H. M. Leicester, *Proc. Soc. Exptl. Biol. Med.*, 77 622 (1951).

Procedure

In the preparation of iodoacetic acid,¹ an aqueous solution of 200 mg. of potassium iodide and 200 mg. of chloroacetic acid, to which has been added 1.75 mc. of carrier-free iodine- I_2^{31} , is heated for 1 hour at 50–60°. Any free iodine is reduced with sodium bisulfite, and the solution is twice extracted with ether. The aqueous layer is treated with 50 mg. of iodoacetic acid and again extracted with ether. The combined ether extracts are evaporated to dryness, yield 48% (average). The freshly prepared solutions give no color with starch.

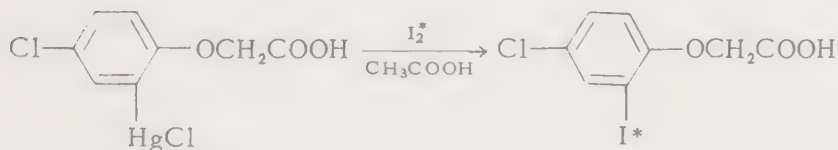
¹E. Abderhalden and M. Guggenheim, *Ber.*, 41, 2853 (1909).

4-IODOPHENOXYACETIC- I^{31} ACID

K. S. Bokarev and H. N. Mel'nikov, *Doklady Akad. Nauk S.S.S.R.*, 97, 255 (1954); through *Chem. Abstracts*, 49, 8846 (1955).

A. Procedure

A solution of 1.62 g. of 4-aminophenoxyacetic acid sulfate, m.p. 186–188° (dec), in 30 ml. of water containing 5 ml. of sulfuric acid is cooled to 0°. During 1 hour, the stirred solution is treated with 0.63 g. of solid potassium nitrite. After the reaction mixture is stirred for an additional hour, it is filtered into a distilling flask which is connected through a trap containing 20% potassium hydroxide to a water-pump. Under reduced pressure, the filtrate is treated with a solution of 1.85 g. of potassium iodide- I^{131} in 10 ml. of water and is then heated on a steam-bath for 2 hours. After the addition of 1 g. of sodium thiosulfate, the solution is cooled to obtain a 48.2% yield of 4-iodophenoxyacetic- I^{131} acid; m.p. 155° from dichloromethane.

(4-CHLORO-2-IODOPHENOXY)ACETIC- I^{131} ACID

R. L. Jones, J. Chem. Soc., 1952, 4080.

A. Procedure

In the all-glass, continuous type apparatus shown in Figure XV, 2 (Note 1), free iodine- I_2^{131} is prepared from a solution of sodium iodide- I^{131} (500 mc.) in 20 ml. of water which also contains 10–20 mg. of sodium sulfite. This solution is transferred from its container in the lead safe A into flask B by applying vacuum *via* I (in the raised position) through C. The original container is rinsed with 5 ml. of water. The vacuum is released and an aqueous solution of 240 mg. of potassium iodide in 5 ml. of water and 5 ml. of 2.76 M sulfuric acid are added *via* tube H. The mixture is shaken and 8 ml. of a potassium dichromate solution (3.02 g. in 250 ml.) is added. The mixture is again shaken and stored at room temperature overnight. A pellet of 0.20 g. of [4-chloro-2-(chloromercuri)phenoxy]acetic acid (Note 2), coarsely powdered and moistened with acetic acid, is frozen in a glass tube. This pellet is added through G into the flask containing the iodine- I_2^{131} and 2.5 ml. of acetic acid is added *via* H. The mixture is heated in a bath at 90° for 1 hour. Then the mixture is cooled; after 20 minutes, 20 ml. of water is added and the mixture is agitated and set aside for 15 minutes. Tube I is lowered and the mother liquor is filtered into C. The residue of mercuric iodide and (4-chloro-2-iodophenoxy)acetic- I^{131} acid is washed with two 5-ml. portions of water and then extracted with 5 ml. of a solution of triethanolamine (Note 3),

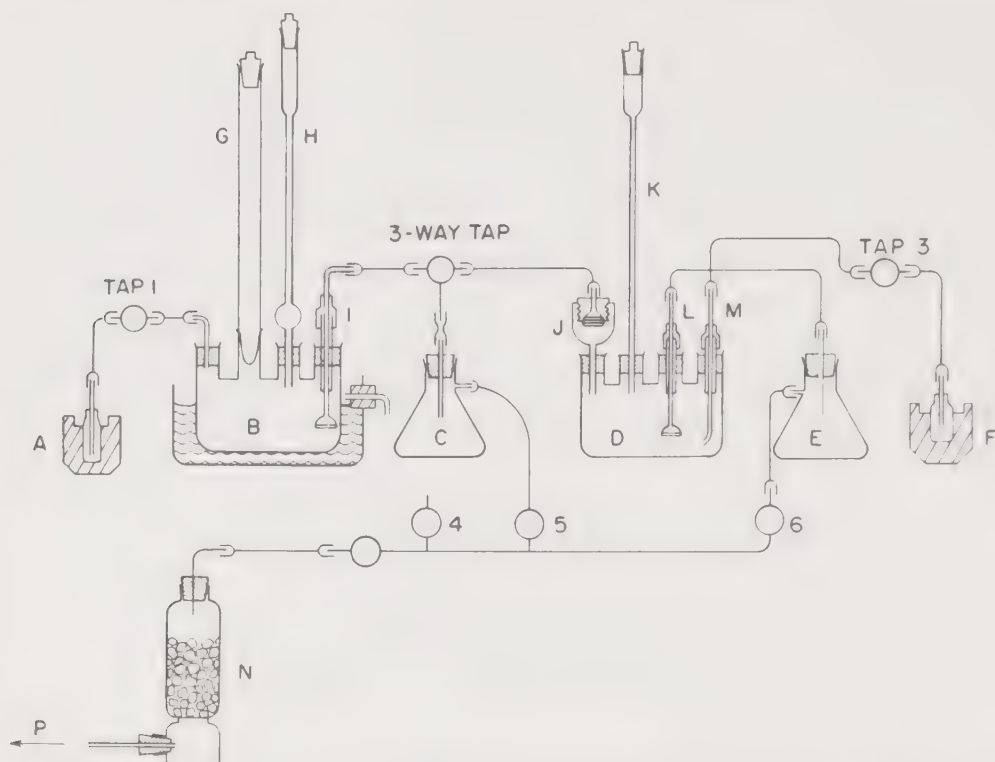


Fig. XV, 2 Apparatus for the preparation of (4-chloro-2-iodophenoxy)acetic- I^{131} acid (R. L. Jones). A and F, lead safes; B and D, 4-necked reaction flasks; C and E, filter flasks; I, J and L, filters; N, scrubbing tower; P, to water aspirator.

with intermittent shaking for 5 minutes. The extract is transferred with vacuum into flask D through filters I and J. The residue in B is washed with two 5-ml. portions of water which are also added to D. Then to the mixture in D is added 5 ml. of dilute hydrochloric acid (Note 4) through tube K. The mixture is shaken, and, after 10 minutes, is filtered through L into flask E. The white solid remaining is washed with two 5-ml. portions of water and is then dissolved in 3 ml. of ethanol (98%) (Note 5). The solvent is evaporated by drawing a stream of dry air through K and L (in the raised position) over the ethanolic solution for 16 hours (Note 6).

B. Notes

1. The apparatus was set up in a hood behind a lead screen. Tubes G, H and K extended above the screen. I and L were 9-mm. diameter sintered glass disc filters of grade 3 porosity which could be raised and lowered in rubber sleeves. Tube M could also be raised and lowered. J was an auxiliary filter made of Perspex and contained four 1-cm. filter papers (Whatman No. 42 grade).

2. The preparation of [4-chloro-2-(chloromercuri)phenoxy]acetic acid, m.p. 189–190°, from 4-chlorophenoxyacetic acid and mercuric acetate is described by Jones.

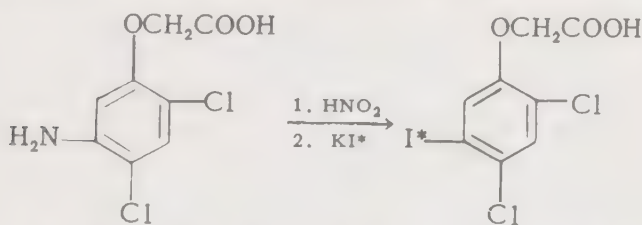
3. This solution contained 20 g. of triethanolamine in 250 ml. of water.

4. Concentrated acid, 30 ml. of d. 1.16, was diluted to 250 ml.

5. Any solid sticking to L was washed off by blowing air through the filter in the lowered position.

6. The residue was redissolved in 1 ml. of 98% ethanol, 40 ml. of olive oil was added and the resulting solution was transferred into the lead safe F. In preliminary runs, the yield of (3-chloro-2-iodophenoxy)-acetic acid, m.p. 139–140°, from 0.20 g. of [4-chloro-2-(chloromercuri)-phenoxy]acetic acid was 0.1 ± 0.01 g.

(2,4-DICHLORO-5-IODOPHENOXY)ACETIC- I^{131} ACID



W. C. Wolfe, J. W. Wood, L. W. Klipp, T. D. Fontaine and J. W. Mitchell, *J. Org. Chem.*, **14**, 900 (1949).

A. Procedure

(a) (2,4-Dichloro-5-iodophenoxy)acetic- I^{131} Acid. The sodium salt of 5-amino-2,4-dichlorophenoxyacetic acid, 11.81 g., is diazotized with sodium nitrite in sulfuric acid at 0–5° (Note 1). A solution of 8.72 g. of potassium iodide- I^{131} in 20 ml. of water is added rapidly with stirring, and the reaction mixture is warmed to 70–80° for 30 minutes. The suspension of crude product is cooled to 15–20° and siphoned with vacuum into a sintered glass funnel which is an integral part of an all-glass purification apparatus (Note 2). The crude product is collected on a filter, washed with 5% sodium bisulfite solution, then with water, and is dissolved in 150 ml. of absolute alcohol heated under reflux. The hot solution is filtered with vacuum into a 500-ml. flask where it is treated with 0.5 g. of activated carbon and again filtered. To the filtrate is added 150 ml. of hot water with thorough mixing. After cooling the mixture, the crude product is collected, mixed with 10 g. of nonisotopic (2,4-dichloro-5-iodophenoxy)acetic acid and recrystallized from 50% ethanol. The yield of product melting at 151.5–152.5° is 18.1 g. (47%) (Note 3).

(b) *Ammonium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . A solution of dry ammonia in ethanol is added to an ethanolic solution of the acid to obtain the ammonium salt, m.p. 234–235°.

(c) *Ethylammonium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . To a solution of the acid in ethanol is added an alcoholic solution of ethylamine. The ethylammonium salt is recrystallized from ethanol, m.p. 194–195°.

(d) *Diethylammonium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The acid and an excess of the amine are reacted at room temperature, the mixture is diluted with ethanol, and the salt is recrystallized from benzene, m.p. 145.5–146.5°.

(e) *Morpholinium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The acid is reacted with an excess of the amine, the mixture is diluted with ethanol, and the salt is recrystallized from ethanol, m.p. 193.5–197.5°.

(f) *Triethanolammonium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . To a solution of the acid in ethanol is added an alcoholic solution of triethanolamine. The salt is recrystallized from ethanol, m.p. 113–114°.

(g) *Sodium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The calculated amount of 1 N sodium hydroxide is added to an alcoholic solution of the acid; the salt is recrystallized from 50% ethanol.

(h) *Potassium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The potassium salt is prepared in the same manner as the sodium salt.

(i) *Calcium (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . An aqueous solution of calcium acetate is added to an ethanol solution of the acid; the calcium salt is recrystallized from 50% ethanol.

(j) *Cupric (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The cupric salt is prepared in the same manner as the calcium salt.

(k) *(2,4-Dichloro-5-iodophenoxy)acetyl- I^{131} Chloride*. The acid is heated under reflux 1.5 hours with an excess of thionyl chloride; the acid chloride is vacuum-distilled, b.p. 180° (5 mm.).

(l) *Methyl (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The acid chloride is heated under reflux with an excess of absolute methanol. The methyl ester is recrystallized from 50% ethanol, m.p. 109.5–111.5°.

(m) *Ethyl (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The ethyl ester is prepared in the same manner as the methyl ester and melts at 83.5–84.5°.

(n) *Isopropyl (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The isopropyl ester is prepared in the same manner as the methyl and ethyl esters.

(o) *Butyl (2,4-Dichloro-5-iodophenoxy)acetate- I^{131}* . The butyl ester, m.p. 70.5–71.0°, is prepared as are the other esters.

(p) *2-(2,4-Dichloro-5-iodophenoxy)acetamide- I^{131}* . The acid chloride is treated with ice-cold concentrated ammonium hydroxide, and the amide is recrystallized from 50% ethanol, m.p. 160–161°.

B. Notes

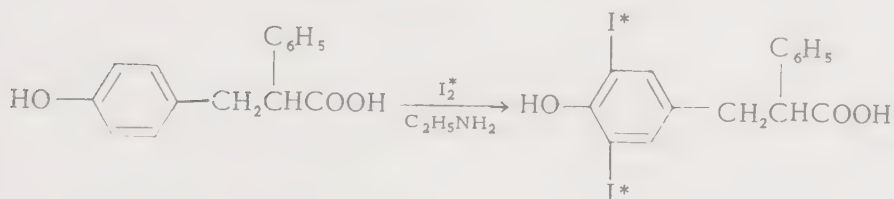
1. Urea is added to destroy excess nitrous acid.

2. A diagram of the apparatus and its manipulation are given by Wolfe, *et al.*

3. Mahler, Speer and Roberts¹ also prepared (2,4-dichloro-5-iodophenoxy)acetic- I^{131} acid, apparently by the procedure of Wolfe, *et al.* They report a much higher yield, 80%, on a 75-mg. scale.

¹H. R. Mahler, R. J. Speer, and A. Roberts, *Science*, **110**, 562 (1949).

3,5-DIIODO- α -PHENYLPHLORETIC- I^{131} ACID (Pheniodol- I^{131})

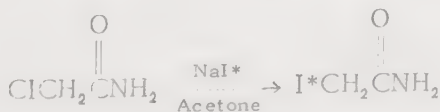


A. A. Free, J. E. Page and E. A. Woollett, *Biochem. J.*, **48**, 490 (1951).

Procedure

An ether solution of iodine- I_2^{131} prepared from 1 mc. of sodium iodide- I^{131} and 10 mg. of inactive sodium iodide is added to a solution of 0.7 g. of α -phenylphloretic acid in 60 ml. of 8% aqueous ethylamine solution, and the mixture is stirred for 15 minutes. The theoretical quantity, 6.3 ml. of 1.9 *N* iodine in excess sodium iodide, is added dropwise during 5 minutes, and stirring is continued for 1 hour. To this mixture then is added 60 ml. of chloroform, the aqueous phase is acidified with hydrochloric acid, and the product passes into the chloroform layer. The chloroform solution is washed once with 10% sodium pyrosulfite, to remove free iodine, and twice with water. It is then concentrated to 15 ml., 30 ml. of light petroleum (b.p. 100–120°) is added, and the product crystallizes. The yield of pheniodol- I^{131} is 1.2 g., m.p. 157–160°.

2-iodoacetamide- I^{131}



O. M. Friedman and A. M. Rutenburg, *J. Am. Chem. Soc.*, **72**, 3285 (1950).

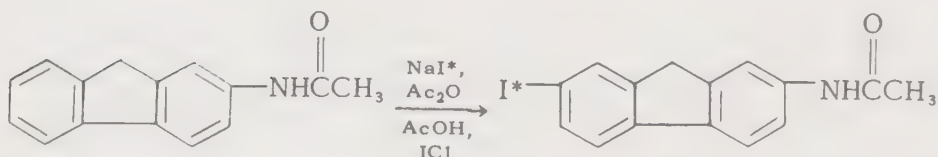
Procedure

A solution of 15 mg. of 2-chloroacetamide¹ and 25 mg. of sodium iodide- I^{131} in 5 ml. of acetone is refluxed for 15 minutes. The precipitate of so-

dium chloride is filtered off, and the solvent is distilled from the filtrate. The residue is crystallized by dissolution in 5 drops of ethyl acetate, which is then cooled in ice and diluted with a few drops of benzin. The supernatant liquid is decanted, and the process is repeated to obtain 15 mg. of white, flaky crystals, m.p. 93-95°.

¹R. Scholl, Ber., 29, 2417 (1896).

***N*-7-iodo-2-fluorenylacetylamine-*I*¹³¹**
(2-Acetamido-7-iodofluorene-*I*¹³¹)



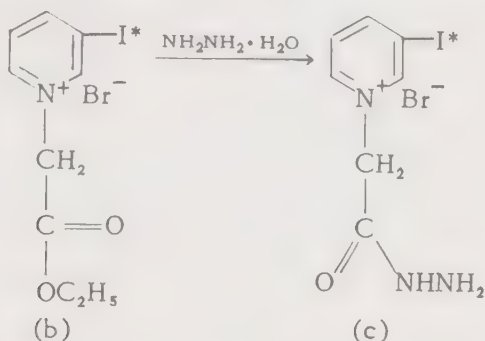
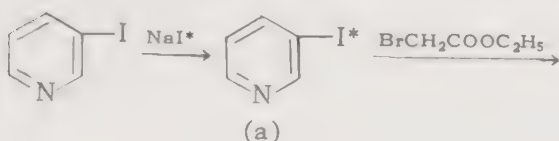
E. K. Weisburger, J. Am. Chem. Soc., 72, 1758 (1950).

A. Procedure

A solution of 2.23 g. of *N*-2-fluorenylacetylamine in 50 ml. of glacial acetic acid is cooled to 22°. A mixture of 5 ml. of sodium iodide-*I*¹³¹ solution (16 μ c.), 5 ml. of acetic anhydride and 2 ml. of glacial acetic acid is allowed to stand for 5 minutes. Then is added 0.75 ml. of iodine monochloride and, after 1 minute, the mixture is added dropwise with stirring, over a period of 5 minutes, to the acetamidofluorene solution. A tan precipitate appears after several minutes. After 7 hours, 100 ml. of water containing 0.5 g. of sodium bisulfite is added slowly, and the mixture is centrifuged. The precipitate is washed 3 times with water by centrifugation. Recrystallization of the precipitate from 70% acetic acid gives 2.0 g. of light tan needles (Note 1), in approximately 75% isotopic yield.

B. Notes

1. Nonisotopic *N*-7-iodo-2-fluorenylacetylamine was prepared by reduction of 2-iodo-7-nitrofluorene, followed by acetylation and by iodination of *N*-2-fluorenylacetylamine with two molar equivalents of iodine monochloride. After recrystallization from ethanol the product melted at 224-225°.

1-(CARBOXYMETHYL)-3-IODOPYRIDINIUM- I^{131} BROMIDE HYDRAZIDE


W. S. Ruliffson, H. M. Lang and J. P. Hummel, *J. Biol. Chem.*, **201**, 839 (1953).

A. Procedure

(a) *3-Iodopyridine- I^{131}* . To 513 mg. of 3-iodopyridine in a 50-ml. flask is added 0.16 mg. of potassium iodide, 0.26 mg. of iodine in 5 ml. of 90% alcohol and 1 ml. of sodium iodide- I^{131} solution (5.64 mc.). Absolute alcohol is added to make the alcohol concentration 90% and, using pH paper, sufficient acetic acid is added to adjust the pH to 5 (Note 1). The mixture is then refluxed gently for a period of 64 hours. The mixture is cooled to room temperature, treated with 1 ml. of concentrated hydrochloric acid and evaporated, with gentle heating, to a volume of about 2 ml. After addition of 3 ml. of water, the mixture is chilled in an ice-bath, and 3-iodopyridine- I^{131} is precipitated by the addition of 2 ml. of 50% potassium hydroxide. After about 30 minutes, the product is collected and washed six times with 2-ml. portions of ice-water (Note 2).

(b) *1-(Carbethoxymethyl)-3-iodopyridinium- I^{131} Bromide*. The 3-iodopyridine- I^{131} is dissolved in 10 ml. of absolute alcohol, 0.54 ml. of ethyl bromoacetate is added, and the solution is refluxed gently for 4 hours. After cooling the solution to 0° for several hours, the pyridinium ester is collected and washed twice with small portions of cold absolute alcohol. The product is recrystallized twice from 13-ml. portions of hot 95% ethanol. Each time the crystallization is completed by the addition of an equal volume of ether (Note 3). The yield of the ester, m.p. 185–188° (dec.), is 681 mg. (76%).

(c) *1-(Carboxymethyl)-3-iodopyridinium- I^{131} Bromide Hydrazide*. The pyridinium ester is dissolved in 27 ml. of anhydrous methanol. This solution, cooled to 5° (Note 4), is then added to a solution of 0.62 ml. of 85% hydrazine hydrate in 2 ml. of absolute methanol also at 5°, and the

mixture is kept in a refrigerator at -20° for 12 hours. Light tan rosettes of the product form on the walls of the vessel, and the yield is increased by concentrating the solution, *in vacuo* without heating, to a volume of about 5 ml. The product is collected on a small suction funnel, washed with cold methanol, and dried on the funnel (Note 5). The yield of product, m.p. $196-198^{\circ}$ with decomposition, is 488 mg. (55%).

B. Notes

1. A study of the exchange reaction indicated a marked dependence upon pH; the optimum appeared to be pH 5.

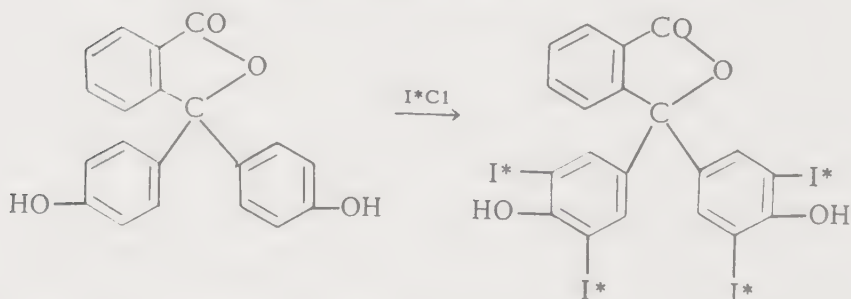
2. Because of the volatility of 3-iodopyridine, it is not weighed at this stage but is immediately converted to the pyridinium ester.

3. The specific activity was usually constant after two recrystallizations.

4. In this reaction undesired impurities form unless the reactants are kept cold throughout the entire reaction period.

5. The product is not hygroscopic.

3',3'',5',5''-TETRAIODOPHENOLPHTHALEIN- I_4^{131} [3,3-Bis(4-hydroxy-3,5-diiodophenyl)phthalide- I_4^{131}]



G. H. Copher, V. H. Wallingford, W. G. Scott, G. G. Zedler, B. Hayward and S. Moore, *Am. J. Roentgenol.*, 67, 964 (1952).

A. Procedure

In a flask equipped with a stirrer, a mixture of 1.130 g. of phenolphthalein and 0.735 g. of tetraiodophenolphthalein is dissolved in 20 ml. of water by the addition of 1.0 ml. of 38% sodium hydroxide solution (wt./vol). Then, 4.5 ml. of 10% hydrochloric acid is added to precipitate the phthaleins, and the mixture is stirred for 15 minutes.

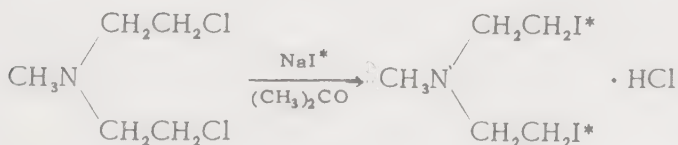
The free iodine- I_2^{131} , from 0.001 g. of sodium iodide- I^{131} (Note 1), is extracted from carbon tetrachloride into 10 ml. of water containing 1.5 ml. of 1 N sodium hydroxide. The solution is acidified with 2.0 ml. of 1 N

hydrochloric acid, and a solution of 0.92 ml. of iodine monochloride in 1 ml. of concentrated hydrochloric acid is added. The combined solution is added immediately to the flask containing the phthaleins, and the mixture is stirred for 13.5 hours at room temperature. At this time, 0.5 g. of sodium bisulfite is added to the mixture, and the crude tetraiodophenolphthalein- I_4^{131} is dissolved by the addition of 7.0 ml. of 38% sodium hydroxide solution. The product is reprecipitated by the addition of 7.5 ml. of dilute (1:5) acetic acid, and the mixture is stirred for 30 minutes at 70–90° to coagulate the product. The 3',3'',5',5''-tetraiodophenolphthalein- I_4^{131} is collected on a sintered glass funnel using vacuum and washed several times with distilled water (Note 2).

B. Notes

1. The sodium iodide- I^{131} solution was treated with nitrous acid, and the isotope was extracted into carbon tetrachloride.
2. Assay of the final product indicated that 76.7% of the total activity was incorporated into the 3',3'',5',5''-tetraiodophenolphthalein- I_4^{131} .

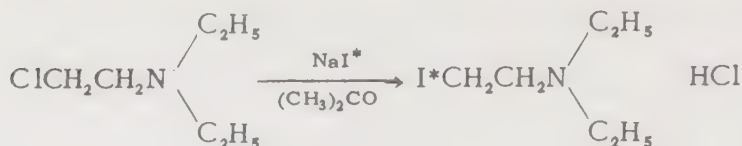
2,2'-DIIDO-N-METHYLDIETHYLAMINE- I_2^{131} HYDROCHLORIDE



A. M. Seligman, O. M. Friedman, and A. M. Rutenburg, *Cancer*, 3, 343 (1950).

A. Procedure

A solution of 23 mg. of 2,2'-dichloro-*N*-methyldiethylamine and 50 mg. of sodium iodide- I^{131} in 2 ml. of acetone is heated in a sealed tube at 125° for 30 minutes. The mixture is filtered to remove sodium chloride and evaporated to dryness. The residue is extracted with warm petroleum ether (total 10 ml.), which is separated by decantation. Upon treatment with dry hydrogen chloride, the solution becomes milky and deposits a gummy solid after about 15 minutes. The petroleum ether is decanted; the residue is washed with a small amount of ethyl acetate, which removes the gummy impurities, and is transferred to a filter. There is obtained 26 mg. (20%) of the amine hydrochloride, m.p. 180° (sinters at 124°).

2-IODOTRIETHYLAMINE- I^{131} HYDROCHLORIDE

A. M. Seligman, A. M. Rutenburg and O. M. Friedman, J. Natl. Cancer Inst., 9, 262 (1949).

A. Procedure

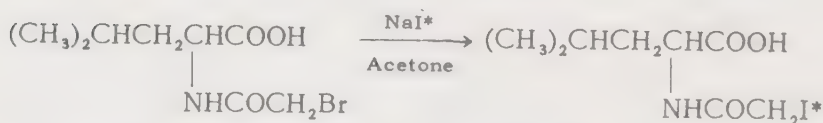
2-Chlorotriethylamine hydrochloride,¹ 0.043 g., is dissolved in reagent acetone, and a solution of 0.050 g. of sodium iodide- I^{131} in 5 ml. of acetone is added. The mixture is warmed on a steam-bath for 8 minutes; precipitation of sodium chloride is complete, and practically all the acetone has evaporated. Dry benzene is added to precipitate any excess sodium iodide, and the mixture is filtered. Hydrogen chloride is bubbled into the filtrate for 3 minutes. After the solution is concentrated on a steam-bath, the amine hydrochloride crystallizes in fine white needles, m.p. 166–167° (Note 1). The yield is 0.060 g. (70% based on sodium iodide- I^{131}).

B. Notes

1. A sample of non-labeled 2-iodotriethylamine hydrochloride was repeatedly recrystallized from alcohol-benzene in white needles, m.p. 173–174°.

¹G. A. C. Gough and H. King, J. Chem. Soc., 1928, 2426.

N-(IODOACETYL)-L-LEUCINE- I^{131}
[2-(2-Iodoacetamido)-5-methylpentanoic- I^{131} Acid]



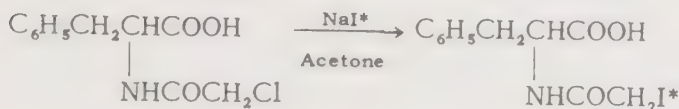
O. M. Friedman and A. M. Rutenburg, J. Am. Chem. Soc., 72, 3285 (1950).

Procedure

A mixture of 44 mg. of *N*-bromoacetyl-L-leucine¹ and 25 mg. of sodium iodide- I^{131} in 10 ml. of reagent acetone is heated under reflux for 10 minutes. The precipitate of sodium bromide is filtered off, the filtrate is diluted with 0.5 ml. of water and heated to evaporate the acetone. The remaining aqueous solution is cooled and seeded to obtain 37 mg. of crystalline product, m.p. 164–165°.

¹E. Abderhalden and W. Zeisset, Ferment., 11, 174 (1930).

N-iodoacetyl-3-phenylalanine-I¹³¹
[2-(2-Iodoacetamido)-3-phenylpropionic-I¹³¹ Acid]



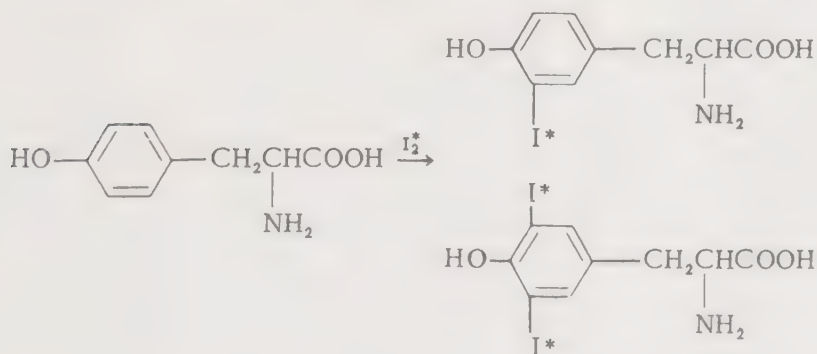
O. M. Friedman and A. M. Rutenburg, J. Am. Chem. Soc., 72, 3285 (1950).

Procedure

A solution of 49 mg. of *N*-chloroacetyl-3-phenylalanine¹ and 35 mg. of sodium iodide-I¹³¹ in 5 ml. of acetone is heated under reflux for 30 minutes. After removal of the sodium chloride precipitate, the reaction mixture is concentrated, diluted with 5 drops of water and further concentrated on a steam-cone to incipient cloudiness. The precipitate obtained on cooling the solution in ice is collected on a filter, washed with a minimum of cold water and dried in a desiccator. The white crystalline product weighs 55 mg., m.p. 137-139°.

¹H. Leuchs and U. Suzuki, Ber., 37, 3313 (1904).

L-3-iodotyrosine-I¹³¹ AND L-3,5-diiodotyrosine-I¹³¹



R. M. Lemmon, W. Tarpey and K. G. Scott, J. Am. Chem. Soc., 72, 758 (1950).

A. Procedure (Note 1)

To an aqueous solution containing 1.72 $\mu\text{equiv.}$ of iodine-I¹³¹ (Note 2) is added 90 $\mu\text{g.}$ of L-tyrosine, dissolved in 20 $\mu\text{l.}$ of concentrated ammonium hydroxide, with stirring (Note 3). To complete the reaction, 84 $\mu\text{g.}$ (0.66 $\mu\text{equiv.}$) of iodine in 20 $\mu\text{l.}$ of 0.174 *N* potassium iodide solution is added to the mixture. Thus, the 3050 $\mu\text{c.}$ of I¹³¹ is distributed in 6.13 $\mu\text{equiv.}$ of triiodide (Note 4). After standing for 6 hours with occasional stirring, the entire solution is transferred to the corner of a large filter

paper and chromatographed (Note 5). The 3-iodotyrosine- I^{131} obtained after elution contains 79 $\mu\text{c.}$ or 1.6% of the starting activity, and the 3,5-diiodotyrosine- I_2^{131} contains 355 $\mu\text{c.}$ or 11.6% of the initial iodine-131 (Note 6). That the starting tyrosine was completely reacted was shown by spraying the paper with ninhydrin; no tyrosine could be detected. From the known specific activity of the iodine used in the reaction (3050 $\mu\text{c.}/6.13 \mu\text{equiv.}$ or 498 $\mu\text{c.}/\mu\text{equiv.}$) the specific activities of the mono- and diiodotyrosine products are known to be 498 and 996 $\mu\text{c.}/\mu\text{mole}$, respectively. From these values and the total activities above, the yields of the two products are calculated to be 48 and 154 $\mu\text{g.}$ respectively. These yields are 32% and 71% of the starting tyrosine and indicate that the iodination is partially complete after 6 hours. A decrease in the starting ratio of iodine to tyrosine leads to a decrease in the yield of diiodotyrosine (Note 7).

B. Notes

1. The method of Harington¹ for the iodination of tyrosine was adapted to a micro-scale preparation.

2. Prepared by iodate oxidation of sodium iodide.

3. The iodine color slowly disappears during this addition.

4. This includes the excess iodate which was reduced to iodide by the bisulfite.

5. The chromatography² of the iodotyrosine reaction mixture was carried out on Schleicher and Schnell filter paper number 589 (23'' \times 23''). Development of the paper in one direction with a butanol-acetic acid-water solvent (74:19:51 parts by volume) and in the other direction with water-saturated phenol gave excellent separations. Further details of the chromatography are given by Lemmons, *et al.*, who also refer to Consden.³ After development of the chromatogram, a radioautograph showed three distinct well-separated spots. Two of the spots (together containing about 15% of the activity on the paper) were near each other in the far corner of the paper; their average R_f values were 0.66 and 0.76 for the butanol-acetic acid solvent. The third spot, not developed nearly as far (R_f value about 0.32), was unused sodium iodide- I^{131} .

The two spots containing organically bound iodine were cut from the paper and eluted separately overnight with about 3 ml. of 50% alcohol. The elution was effected by cutting the paper to a tapered end, suspending the paper downward from a glass trough containing the eluting solvent, and allowing the tapered end to rest against the side of a sloping test tube; the operation was carried out under a glass jar. The elution was followed by the decrease in radioactivity on the paper strip. The identity of the monoiodotyrosine- I^{131} was established by rechromatographing the radioactive material from the lower (R_f value 0.66, butanol-acetic

acid) of the two spots with a sample of known monoiodotyrosine. The radioactivity coincided exactly with the position of the known amino acid as shown by spraying the paper with ninhydrin reagent (0.5% in absolute alcohol), and preparing a radioautograph of the chromatogram. The identity of the diiodotyrosine- I_2^{131} spot was established in exactly the same manner.

In view of the work of Miller,⁴ (see Other Preparations) it is especially of interest that in rechromatographing the pure mono- and diiodotyrosine a small fraction of the active iodine was detached, 2 to 4% for both products.

6. Allowance was made for the radioactive decay of the iodine during the reaction.

7. For example, when the starting ratio of iodine to tyrosine was 2.1, the ratio of diiodotyrosine to monoiodotyrosine was 2.2. When the starting ratio was 1.9, the ratio of products was 1.4.

C. Other Preparations

Of particular significance in the case of metabolic studies is the work of Miller,⁴ who has shown that L-3,5-diiodotyrosine- I_2^{131} exchanges readily with either iodine or iodide ion. The reverse reaction also progresses rapidly. At a diiodotyrosine concentration of 2.2×10^{-6} mole/ml., a diiodotyrosine to radioiodide equivalent ratio of 10 to 1, and a pH of about 5, exchange at 25° is extremely slow, but at 90° is 97% complete in 3 minutes. At 50°, exchanges are observed of 9, 29 and 92% in 5, 15 and 90 minutes, respectively. A modification⁵ of Miller's method⁴ has been used to prepare both L-3,5-diiodotyrosine- I_2^{131} and L-N-acetyl-3,5-diiodotyrosine- I_2^{131} .

Roche⁶ has described the separation of 3-iodotyrosine- I^{131} and 3,5-diiodotyrosine- I_2^{131} from thyroglobulin hydrolysates by paper chromatography on Whatman No. 1 paper, developed with a mixture containing 68% butanol, 5% acetic acid and 27% water.

A study⁷ has been made of the iodination of tyrosine and histidine with the concentration of iodine ranging from 0.4 to 15.0 atoms per molecule of amino acid. In the case of tyrosine a mixture of 3-iodotyrosine- I^{131} and 3,5-diiodotyrosine- I_2^{131} results until the concentration of iodine reaches 6 atoms per molecule of amino acid, above which only 3,5-diiodotyrosine- I_2^{131} results. In the case of histidine, the products are 2-iodohistidine- I^{131} and 2,5-diiodohistidine- I_2^{131} , and the limiting concentration of iodine for the formation of monoiodohistidine is 8 atoms per molecule of amino acid.

¹C. R. Harington, *Biochem. J.*, **38**, 320 (1944).

²R. Consden, A. H. Gordon and A. J. P. Martin, *Biochem. J.*, **38**, 224 (1944).

³R. Consden, *Nature*, **162**, 359 (1948).

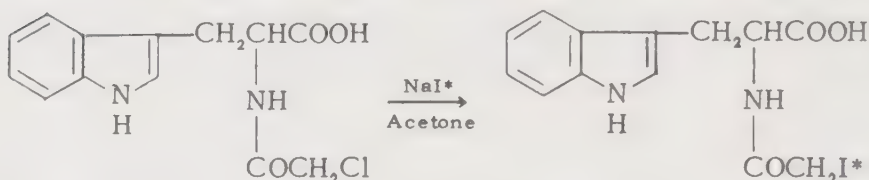
⁴W. H. Miller, G. W. Anderson, R. K. Madison and D. J. Salley, *Science*, **100**, 340 (1944).

⁵W. Tong, A. Taurog and I. L. Chaikoff, J. Biol. Chem., 207, 59 (1954).

⁶J. Roche, M. Jutisz, S. Lissitzky and R. Michel, Compt. rend., 231, 723 (1950).

⁷J. Roche, S. Lissitzky, O. Michel and R. Michel, Biochim. and Biophys. Acta, 7, 439 (1951).

***N*-IODOACETYL-L-TRYPTOPHAN-*I*¹³¹**
[α -(2-Iodoacetamido)-3-indolepropionic-*I*¹³¹ Acid]



O. M. Friedman and A. M. Rutenburg, J. Am. Chem. Soc., 72, 3285 (1950).

A. Procedure

A mixture of 44 mg. of *N*-chloroacetyl-L-tryptophan¹ and 25 mg. of sodium iodide-*I*¹³¹ (Note 1) in 10 ml. of reagent acetone is heated under reflux for 15 minutes. After the precipitate of sodium chloride is removed by filtration, the filtrate is diluted with 0.5 ml. of water and evaporated until it becomes cloudy. From the cold solution is obtained 39 mg. of white, flaky crystals, m.p. 180-182°. After recrystallization from methanol and water, the m.p. is 185-187° (Note 2).

B. Notes

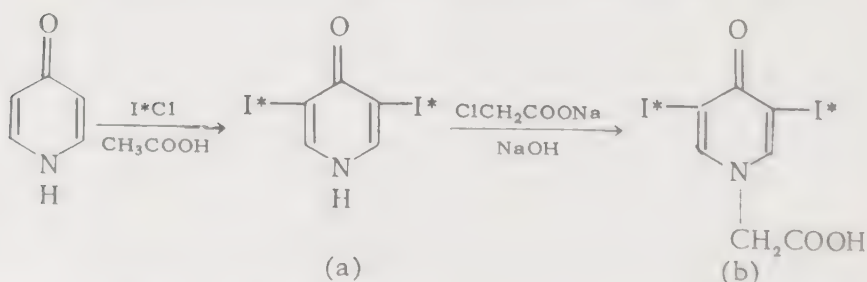
1. The sodium iodide-*I*¹³¹ is prepared by evaporating a solution of sodium iodide-*I*¹³¹ (carrier-free) and the required amount of inactive sodium iodide.

2. A macro preparation by this method gave a product melting at 186-188°; Abderhalden and Baumann² reported 175-176°.

¹E. Abderhalden and M. Kempe, Ber., 40, 2737 (1907).

²E. Abderhalden and L. Baumann, Ber., 41, 2857 (1908).

3,5-DIIODO-4-OXO-1(4*H*)-PYRIDINEACETIC-*I*₂¹³¹ ACID



A. Roe, R. L. Hayes and H. D. Bruner, J. Mitchell Soc., 66, 163 (1950).

A. Procedure

(a) *3,5-Diiodo-4(1H)-pyridone-I₂¹³¹*. Exactly 1.100 g. of dichloramine-T¹ is placed in a 250-ml. flask and dissolved in 30 ml. of hot glacial acetic acid (Note 1). The solution is cooled to room temperature, 1.38 g. of potassium iodide is added, and the liquid is swirled until the iodide dissolves and the orange color of iodine monochloride appears. The desired quantity of sodium iodide-I¹³¹ is added (in 3-4 ml. of water) (Note 2) followed by 0.65 g. of 4(1H)-pyridone (Note 3). During the above additions, a further 10 ml. of glacial acetic acid is used to wash down the neck of the flask (Note 4). A glass-enclosed magnetic stirrer is introduced; the flask, fitted with a Liebig condenser, is placed in a heating mantle, and the contents are refluxed with rapid stirring. Precipitation of 3,5-diiodo-4(1H)-pyridone-I₂¹³¹ soon begins, and reflux, with stirring, is continued for 5 hours, after which time the reaction mixture is colorless. After adding 46 ml. of water and cooling the flask in an ice-bath for 30 minutes, the mother liquor is removed from the product with a filter stick. The residue of 3,5-diiodo-4(1H)-pyridone-I₂¹³¹ is washed three times with ice-water using the filter stick, which is finally rinsed off with 10 ml. of water.

(b) *3,5-Diiodo-4-oxo-1(4H)-pyridineacetic-I₂¹³¹ Acid* (Note 5). To the above aqueous mixture is added 2 ml. of water containing 0.57 g. of sodium hydroxide, and the 3,5-diiodo-4(1H)-pyridone-I₂¹³¹ is dissolved by heating. The flask is fitted with an adapter holding a condenser and an addition funnel. When the solution reaches the boiling point, 0.71 g. of chloroacetic acid is added with vigorous stirring, and the addition funnel is rinsed with a few ml. of water. The solution is refluxed for 1.5 hours and then, while the solution is still hot, 1.75 ml. of water is added with vigorous stirring. After crystallization begins, the flask is cooled for 30 minutes in an ice-bath. The mother liquor is removed with a filter stick, and the product is washed three times with a minimum amount of ice-water. Approximately 45 ml. of water and 0.1 g. of charcoal are added, and the solvent is refluxed to dissolve the product and then filtered through two thicknesses of filter paper. The filtrate is cooled in an ice-bath, and the product is collected in a sintered glass funnel with the aid of a little ice-water. After drying at 110° for 2.5 hours and cooling in a desiccator, the yield of purified product is 73.5% based on the sodium iodide-I¹³¹.

B. Notes

1. The ratio of dichloramine-T to sodium iodide-I¹³¹ was found to be important. An excess of dichloramine-T was necessary to prevent decomposition of iodine monochloride during the reaction with 4-pyridone; but too great an excess resulted in chlorination of the 4-pyridone. The

highest yield of pure 3,5-diiodo-4(1*H*)-pyridone- I_2^{131} was obtained using 1.1 times the stoichiometric amount of dichloramine-T.

2. Addition of the sodium iodide- I^{131} before the inactive potassium iodide carrier lowered the yields, based on iodide- I^{131} , by about 50%.

3. The use of an excess of 4-pyridone, over that required to react with the iodine monochloride present, proved advantageous. The optimum ratio of these reagents was found to be 1.66.

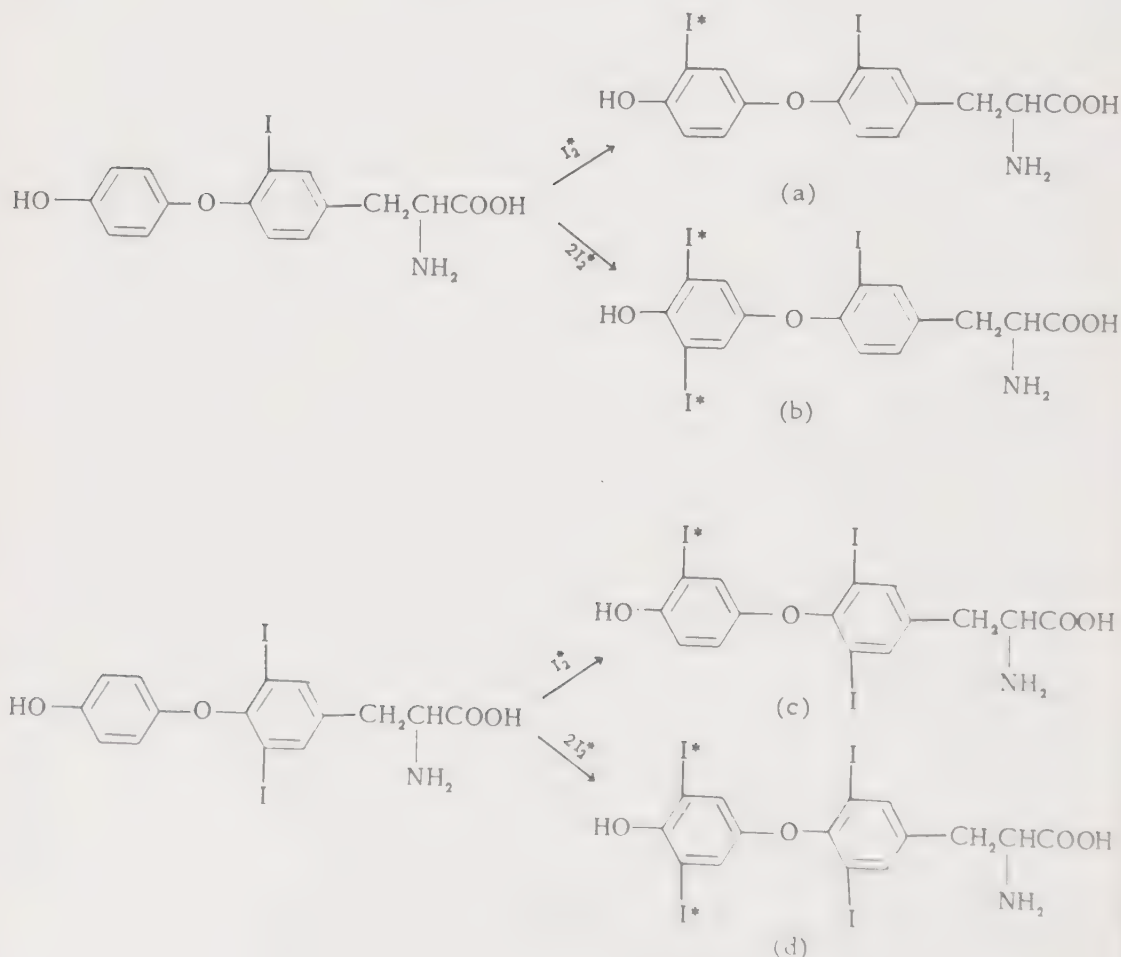
4. The optimum volume ratio of water to acetic acid in the solvent was found to be between the limits of 0.075 and 0.100. Apparently chlorination occurs in glacial acetic acid.

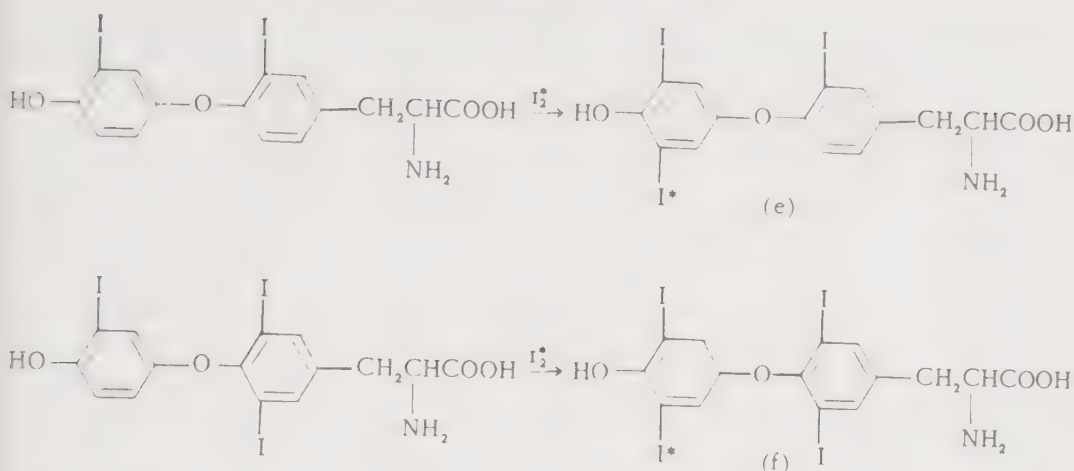
5. This procedure is an adaptation of the method of Baker and Briggs.²

¹R. B. Krauss, Am. J. Pharm., 90, 16 (1918).

²W. Baker and A. S. Briggs, J. Soc. Chem. Ind., 62, 189 (1943).

3-[4-(4-HYDROXY-3-IODOPHENOXY- I^{131})-3-IODOPHENYL]ALANINE (3,3'-Diiodothyronine-3'- I^{131})





J. Roche, R. Michel, P. Jouan and W. Wolf, *Bull. soc. chim. biol.*, 37, 819 (1955).

A. Procedure

(a) 3-[4-(4-Hydroxy-3-iodophenoxy- I^{131})-3-iodophenyl]alanine, (3,3'-Di-iodothyronine-3'- I^{131}). A solution of iodine- I_2^{131} is prepared by exchange, at pH 8, from an alcoholic solution of iodine and an aqueous solution of sodium iodide- I^{131} , free of reducing agents. The exchange reaction is carried out in a bent micropipet, A, with a 50- μl . capacity and with the delivery tip drawn out extremely fine (Figure XV, 3) so that it can be introduced into the capillary extremity of a hematologic pipet. By means of a very fine capillary a drop of sodium iodide- I^{131} is introduced into the bulb of the micropipet, and the pH is adjusted to near 5 by addition of 1 mg. of 0.01 *N* sulfuric acid solution. Then 10 μl . of an iodine solution, containing 52.2 mg. of iodine in 20 ml. of ethanol, is introduced. The solutions are mixed in the reservoir, a, of the micropipet and used as quickly as possible (Note 2).

A solution of 1.81 mg. of 3-iodothyronine¹ in 1.80 ml. of concentrated ammonium hydroxide is prepared. Then 9 μl . of this solution is drawn into the bulb of a carefully calibrated hematologic pipet, B, by means of



Fig. XV, 3 Microapparatus for the preparation of 3,3'-diiodothyronine-3'- I^{131} (J. Roche, R. Michel, P. Jouan and W. Wolf). A, micropipet; B, hematologic pipet; C, syringe.

the attached syringe, C. The capillary, b, of the pipet is rinsed with distilled water to remove ammonia (Note 3), and 9 μ l. of the iodine- I_2^{131} solution (7.1×10^{-2} μ mole) is also drawn into the pipet. The two solutions are carefully mixed and left in the pipet for 15 minutes.

The reaction mixture, which now contains 3,3'-diiodothyronine-3'- I^{131} , is deposited directly from the pipet onto a sheet of Whatman No. 1 filter paper (55 cm. \times 30 cm.) (Note 4) in a thin line 10 cm. in length. The paper, thus prepared, is placed in an electrophoresis tray and soaked with 0.2 M ammonium carbonate buffer (pH 9.5) (Note 5) in a symmetrical pattern around the line of application (Note 6). The electrophoresis is then continued for 150 minutes at 120 volts and 10 milliamperes in the presence of the ammonium carbonate buffer (Note 7). After the paper is dried, the position of the iodides- I^{131} is found by means of a radioautograph, and this portion of the paper is cut off.

The iodothyronines on the remaining paper are separated chromatographically with the solvent system *t*-amyl alcohol saturated with 2 N ammonium hydroxide in ascending phase (Note 8). Following the chromatographic separation, the position of the 3,3'-diiodothyronine-3'- I^{131} is found by means of a new radioautograph and the R_f values for 3-iodothyronine, 3,3'-diiodothyronine and 3,3',5'-triiodothyronine. This zone is eluted from the paper, held between 2 microscope slides, with 1-butanol saturated with water (Note 9).

The solvent is removed under vacuum at a temperature $<30^\circ$, and the residue is taken up in a microdrop of 0.1 N sodium carbonate to which is then added 0.2 ml. of water.

Other iodothyronines prepared in an analogous manner are listed below. In the preparation of (b) or (d) the amount of iodine required is twice that in the synthesis described above; therefore, in order to keep the total volume in the reaction constant, the concentration of iodine in the initial alcoholic solution is doubled.

- (b) 3-[4-(4-Hydroxy-3,5-diiodophenoxy- I_2^{131})-3-iodophenyl]alanine, (3,3',5'-Triiodothyronine-3',5'- I_2^{131})
- (c) 3-[4-(4-Hydroxy-3-iodophenoxy- I^{131})-3,5-diiodophenyl]alanine, (3,3',5'-Triiodothyronine-3'- I^{131})
- (d) 3-[4-(4-Hydroxy-3,5-diiodophenoxy- I_2^{131})-3,5-diiodophenyl]alanine, (Thyroxine-3',5'- I_2^{131})
- (e) 3-[4-(4-Hydroxy-3,5-diiodophenoxy-3- I^{131})-3-iodophenyl]alanine, (3,3',5'-Triiodothyronine-3'- I^{131})
- (f) 3-[4-(4-Hydroxy-3,5-diiodophenoxy-3- I^{131})-3,5-diiodophenyl]alanine, (Thyroxine-3'- I^{131})

B. Notes

1. The micropipet is calibrated before use.
2. It is desirable to keep the solution at a temperature of 10° .

3. The wash liquid is not allowed to come in contact with the solution of iodothyronine but is made to flow back out of the capillary.

4. Papers of these dimensions were chosen to permit electrophoretic separation of iodide ions and the subsequent chromatographic separation of the various thyronines on the same sheet.

5. Ammonium carbonate buffer gave a more rapid migration and cleaner separation of iodide than was found with veronal buffer or volatile buffers such as ammonium acetate.

6. Diffusion was avoided in this manner.

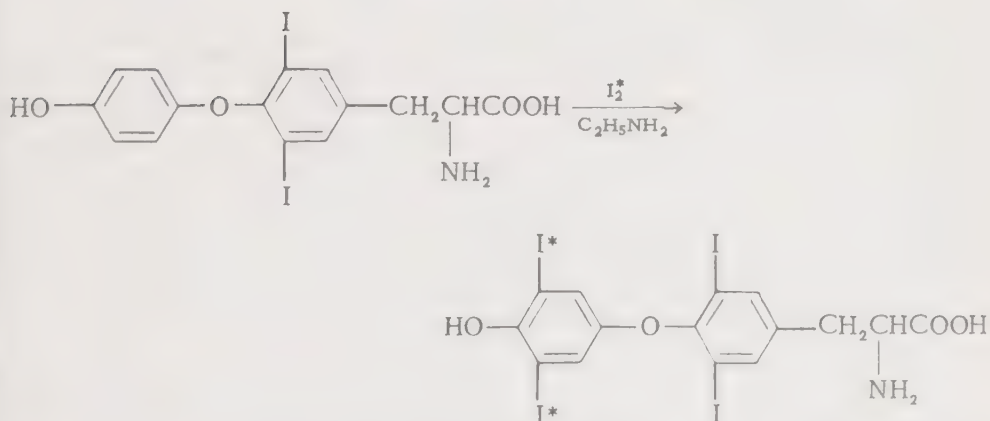
7. The ammonium carbonate buffer assured excellent stability of the voltage even during long periods of electrophoresis and did not impede the ultimate chromatographic separation of the iodothyronines.

8. This solvent does not give good separation of iodides from the iodothyronines but does give good separation of the latter following the removal of the iodides by electrophoresis.

9. From 2 to 3 ml. of solvent is usually sufficient.

¹J. Roche, R. Michel and W. Wolf, *Compt. rend.*, 239, 597 (1954).

3-[4-(4-HYDROXY-3,5-DIIODOPHENOXY- I_2^{131})-3,5-DIIODOPHENYL]ALANINE
(Thyroxine-3',5'- I_2^{131})



J. C. Clayton, A. A. Free, J. E. Page, G. F. Somers and E. A. Woollett, *Biochem. J.*, 46, 598 (1950).

A. Procedure

Iodine-labeled thyroxine is prepared from 3,5-diiodothyronine by a modification of the method of Clayton and Hems.¹ A solution of iodine- I_2^{131} in ether is prepared from 1 mc. of sodium iodide- I^{131} and 2.3 mg of inactive sodium iodide. 3,5-Diiodothyronine, 2 mg., in 0.06 ml. of 33% aqueous ethylamine solution is added, and the mixture is shaken care-

fully until the iodine color is discharged. The ether layer is evaporated with a stream of air, and the aqueous layer is diluted with 1 ml. of water and adjusted to pH 4-5 by adding glacial acetic acid. The suspended thyroxine precipitate is centrifuged and washed with successive 1-ml. amounts of distilled water until the washings contain less than 5% non-thyroxine I^{131} (Note 1). The thyroxine-3',5'- I_2^{131} amounts to approximately 2 mg. (Note 2).

B. Notes

1. This is demonstrated by butanol extraction of the aqueous washings according to Blau;² about 14 washings are required.

2. The crude thyroxine-3',5'- I_2^{131} is dissolved in 10 ml. of 0.02 N sodium carbonate solution and assayed for thyroxine polarographically.³ The product contains at least 90% of thyroxine; the remainder is 3,5-diiodothyronine.

In order to minimize the possibility of exchange between I^{131} and I^{127} , all the thyroxine-3',5'- I_2^{131} solutions and extracts are kept at an alkaline pH. Miller⁴ and Frieden⁵ have shown that exchange between iodide and either 3,5-diiodotyrosine or thyroxine proceeds rapidly at pH 5. Exchange is negligible in alkaline solutions.

C. Other Preparations

Thyroxine-3',5'- I_2^{131} has been prepared: in 16.1% yield,⁶ based on the initial activity, by iodination of 3,5-diiodothyronine in ammoniacal aqueous medium; as the sodium salt by a similar procedure⁷ and in a unique microsynthesis.⁸ Frieden and co-workers⁵ have prepared labeled thyroxine in 30% yield by exchange with iodide- I^{131} in a butanol-water medium at pH 5. There is some question about the location of the isotope in a reaction of this type. Also using the exchange reaction described in Frieden,⁵ Lemmon, *et al.*,⁹ prepared I_2^{131} -thyroxine on a μ g.-scale in 83-89% yield and purified the product by chromatography. The product contained 11% of the starting iodine isotope.

I_1^{131} -Thyroxine has been prepared¹⁰ in 29% isotopic yield by the treatment of triiodothyronine with iodine- I_2^{131} in aqueous diethanolamine. The purified product did not contain any radioactive triiodothyronine, and the only contaminant was a trace of iodide ion.

¹J. C. Clayton and B. A. Hems, *J. Chem. Soc.*, 1950, 840.

²N. F. Blau, *J. Biol. Chem.*, 110, 351 (1935).

³E. T. Burrows, J. C. Clayton, and B. A. Hems, *J. Chem. Soc.*, 1949, S199.

⁴W. H. Miller, G. W. Anderson, R. K. Madison and D. J. Salley, *Science*, 100, 340 (1944).

⁵E. Frieden, M. B. Lipsett and R. J. Winzler, *Science*, 107, 353 (1948).

⁶A. Horeau and P. Süe, *Bull. soc. chim. biol.*, 27, 485 (1945).

⁷J. Gross and C. P. Leblond, *J. Biol. Chem.*, 184, 489 (1950).

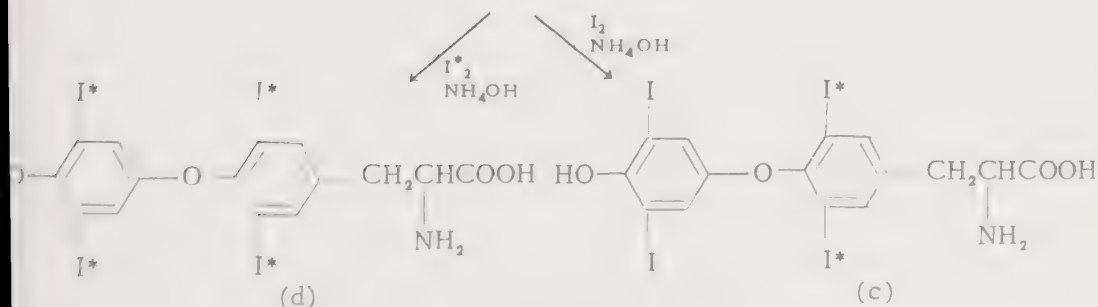
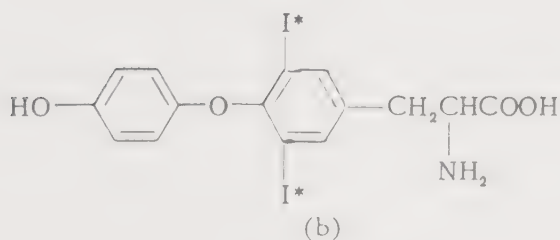
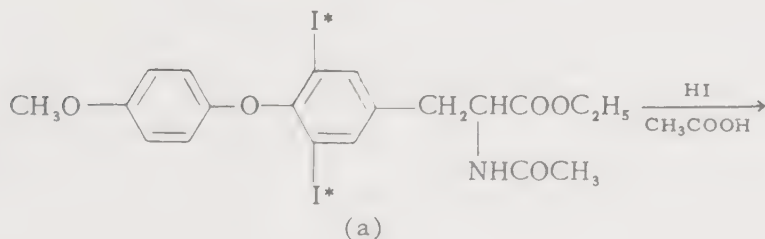
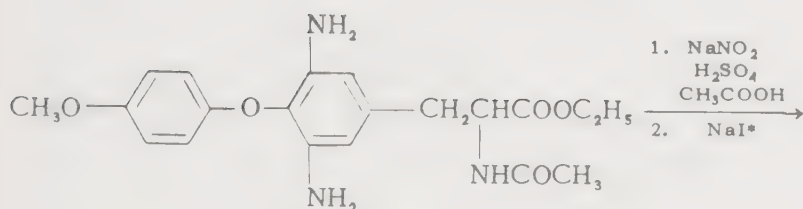
⁸J. Roche, R. Michel, P. Jouan and W. Wolf, Bull. soc. chim. biol., 37, 819 (1955).

⁹R. M. Lemmon, W. Tarpey and K. G. Scott, J. Am. Chem. Soc., 72, 758 (1950).

¹⁰A. Critchlow and M. K. Goldfinch, Proceedings Radioisotope Conference. Volume I, Academic Press Inc., Publishers, New York, 1954, p. 271.

3-[4-(4-HYDROXY-3,5-DIIODOPHENOXY)-3,5-DI
DIODOPHENYL]ALANINE-I₄¹³¹
(Thyroxine-I₄¹³¹)

METHOD I



R. Michel, J. Roche and J. Tata, Bull. soc. chim. biol., 34, 466 (1952).

A. Procedure (Note 1)

(a) *Ethyl N-Acetyl-3-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]alanine- I_2^{131}* . To a solution of 175 mg. of sodium nitrite in 1.2 ml. of concentrated sulfuric acid is added cautiously, with stirring, 2.5 ml. of acetic acid. With the temperature of this solution maintained between -2° and -5° in an ice-salt bath, a solution of 400 mg. of ethyl *N*-acetyl-3-[3,5-diamino-4-(4-methoxyphenoxy)phenyl]alanine in 0.8 ml. of acetic acid, containing 0.4 ml. of concentrated sulfuric acid, is added dropwise with stirring. After this solution is stirred for 1 hour at 0° , a solution of 800 mg. of sodium iodide- I^{131} , 670 mg. of iodine and 100 mg. of urea, in 13 ml. of water, and 2.5 ml. of chloroform are added rapidly. This mixture is heated for 1 hour on a water-bath at 40° . The chloroform phase is separated, and the aqueous phase is extracted with two 3-ml. portions of chloroform. The combined extracts are washed with 5 ml. of water and 3 ml. of solution containing 100 mg. of sodium metabisulfite (Note 2). The chloroform solution is again washed with water and then evaporated. The residue is crystallized from 95% alcohol to obtain 255 mg. of product, m.p. 141° (Note 3).

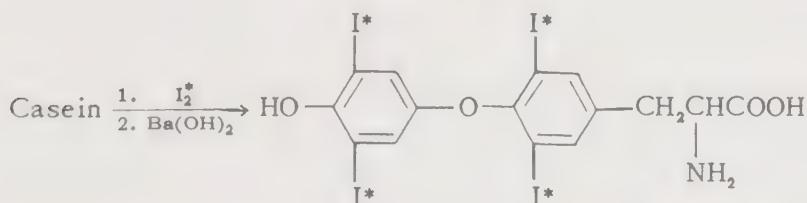
(b) *3-[4-(4-Hydroxyphenoxy)-3,5-diiodophenyl]alanine- I_2^{131} , (3,5-Diiodo-L-thyronine- I_2^{131})*. A mixture of 80 mg. of ethyl *N*-acetyl-3-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]alanine- I_2^{131} , 1.6 ml. of 57% hydriodic acid (Note 4) and 1.6 ml. of acetic acid in a sealed tube is heated in an oil-bath at $130-135^\circ$ for 4 hours. After the tube is cooled and opened, the mixture is heated at 60° under vacuum to remove volatile acids. The residue is dissolved in 0.6 ml. of 95% alcohol at the boiling point, and 0.4 ml. of solution containing 120 mg. of sodium acetate trihydrate and 4 mg. of sodium bisulfite is added with caution. When the solution is cooled, a crystalline precipitate forms which is washed with a few drops of 95% alcohol and dried. The product is then dissolved in the least possible amount of a 10% solution of hydrogen chloride in alcohol; the solution is treated with a small amount of carbon, heated to boiling and centrifuged. Water is added dropwise to the supernatant liquid until a turbidity persists. The product crystallizes in fine needles, m.p. 245° (dec.) (Note 5).

(c) *3-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl- I_2^{131}]alanine, (Thyroxine-3,5- I_2^{131})*. To a solution of 1 mg. of 3,5-diiodothyronine- I_2^{131} in 1 ml. of concentrated ammonium hydroxide is added a solution of 1.5 mg. of iodine in 2 ml. of ether (Note 6). With occasional shaking, the mixture is kept for 1 hour at room temperature. The ether is evaporated, and the remaining aqueous solution is placed on a sheet of Whatman No. 1 paper 40 cm. in length. At other points on the sheet are placed 1 microdrop of each of four solutions containing, respectively: 3,5-diiodothyronine, 3,3',5-triiodothyronine, thyroxine and iodine. Using

isopropyl alcohol saturated with 6 *N* ammonium hydroxide as the solvent, diiodothyronine, triiodothyronine and thyroxine are separated satisfactorily; their R_f values are respectively: 0.38, 0.27 and 0.15. The band on the chromatogram containing the thyroxine-3,5- I_2^{131} (Note 7) is eluted with 4 *N* ammonium hydroxide. The solution is concentrated under vacuum to obtain 1.2 mg. of thyroxine-3,5- I_2^{131} .

(d) 3-[4-(4-Hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl] alanine- I_4^{131} , (Thyroxine- I_4^{131}). By using an ether solution of iodine- I_2^{131} in the above procedure, thyroxine labeled in all four iodine atoms is obtained.

METHOD II



E. Frieden, M. B. Lipsett and R. J. Winzler, *Science*, 107, 354 (1948).

A. Procedure

The iodination of tyrosine-containing proteins¹ is a convenient method of preparing thyroxine with identical specific activity in all four iodinated positions. Casein, 50 g., is suspended in 2 l. of distilled water containing 0.7 per cent sodium bicarbonate. The water is heated to 40° with continuous stirring, and finely powdered iodine, 12 g. containing iodine- I_2^{131} , is added in portions during 4 hours. When all the iodine has combined with the casein, the temperature is increased to 70° where it is maintained, with continuous stirring, for 20 hours.

The iodinated protein is precipitated by the addition of dilute hydrochloric acid until the pH of the solution is about 4. The casein is partially purified by reprecipitation from sodium hydroxide solution with hydrochloric acid. The process is repeated twice; the casein is recovered by filtration, dried and ground to a fine powder.

Thyroxine is prepared from the iodinated casein by a procedure similar to that described by Ludwig and von Mutzenbecher.² A mixture of 50 g. of the protein, 320 ml. of water and 160 g. of barium hydroxide octahydrate is heated, with stirring, until all the barium hydroxide dissolves. The mixture is boiled gently under reflux for 20 hours. The precipitate of barium salts is collected on a filter; the precipitate (A) and the filtrate (B) are then treated separately. Precipitate A is treated with dilute hydrochloric acid, with vigorous stirring, until the mixture is slightly acid to Congo red, then is filtered with vacuum and washed with dilute acetic acid. Filtrate B is cooled, the crystalline barium hydroxide

is collected, redissolved and crystallized again, and the second filtrate is combined with the first. The combined filtrate is acidified to pH 4.5 to 5.0; a light-colored precipitate forms, which is recovered by centrifugation, washed several times with dilute acetic acid, dried and added to precipitate A. The combined, dry precipitate is extracted with three portions of ether and dissolved in 0.1 *N* sodium hydroxide containing a small amount of sodium sulfate, and the solution is heated to boiling. The solution is centrifuged (Note 8) repeatedly until a clear solution is obtained. The solution, heated to 90°, is acidified with dilute sulfuric acid until a granular precipitate forms (pH 5). The precipitate is collected, washed with dilute acetic acid, and dried *in vacuo* over magnesium perchlorate.

The acid-insoluble material is dissolved in a minimum amount of 0.1 *N* sodium carbonate solution, centrifuged (Note 9) and then cooled to 0°. A heavy white precipitate of the monosodium salt of thyroxine- I_4^{131} is formed. The precipitate is collected by centrifugation and is purified by several recrystallizations from 0.1 *N* sodium carbonate solution (Note 10). The salt is dissolved in alkaline 70% alcohol; the solution is heated to boiling and acidified with acetic acid. Thyroxine- I_4^{131} separates immediately in characteristic bundles of microscopic needles. Further purification is effected by a second crystallization from 0.1 *N* sodium carbonate and finally from alkaline alcohol. The yield of thyroxine- I_4^{131} is 0.385–0.424 g. per 100 g. of iodinated casein (Note 11).

B. Notes

1. In addition to the procedure given, the authors have described a similar procedure for the preparation of compounds (a) and (b) with a high specific activity and on a smaller scale.
2. This is used to remove the excess iodine.
3. The chromatographic R_f values found for ethyl *N*-acetyl-3-[3,5-diamino-4-(4-methoxyphenoxy)phenyl]alanine, iodide ion and ethyl *N*-acetyl-3-[3,5-diiodo-4-(4-methoxyphenoxy)phenyl]alanine- I_2^{131} with 1-butanol saturated with 2 *N* ammonium hydroxide, were respectively: 0.05, 0.20 and 0.90.
4. The hydriodic acid is freshly distilled from red phosphorus.
5. Using 1-butanol saturated with 2 *N* ammonium hydroxide, the R_f values found for tyrosine, iodine, thyronine and 3,5-diiodothyronine- I_2^{131} were respectively: 0.06, 0.28, 0.54 and 0.58.
6. According to Roche,³ a minimum of 3 moles of iodine per mole of diiodothyronine is essential to prevent the formation of triiodothyronine.
7. The positions on the chromatogram of the nonradioactive reference compounds were revealed by spraying them with ninhydrin.
8. The barium sulfate is eliminated in this manner.

9. To remove traces of undissolved, dark-colored substance.
10. Additional amounts of the sodium salt are obtained by successively concentrating the mother liquors and recrystallizing the white precipitate that forms upon cooling.
11. Thyroxine decomposes at 231–233°.

C. Other Preparations

I^{131} -Iodocasein has been prepared⁴ by a procedure similar to that described. A 2.5% solution of casein in 1% sodium bicarbonate, containing 10 ml. of colloidal oxides of manganese per gram of casein, was agitated at 70° for 18–20 hours with elemental iodine- I_2^{131} . The product, precipitated at pH 4.6 from the reaction mixture, was washed twice with acetate buffer at pH 4.6, once with alcohol, and dried.

A radiochemical method for the quantitative estimation of micro quantities of thyroxine has been described.⁵ The method makes use of the exchange reaction between thyroxine and tracer amounts of iodine- I_2^{131} in a buffered solution at pH 4.6 and a temperature of 65°. The reaction is catalyzed by elemental iodine.

¹E. P. Reineke and C. W. Turner, *J. Biol. Chem.*, **149**, 555 (1943).

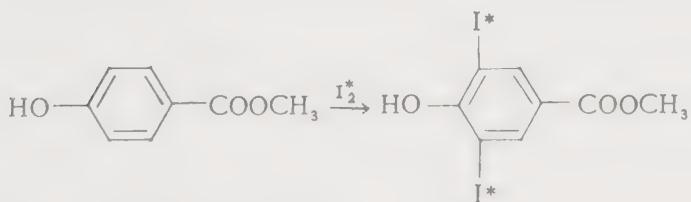
²W. Ludwig and P. von Mutzenbecher, *Z. physiol. Chem.*, **258**, 195 (1939).

³J. Roche, S. Lissitzky and R. Michel, *Compt. rend.*, **234**, 997 (1952).

⁴C. F. Hamilton, M. H. Power and A. Albert, *J. Biol. Chem.*, **178**, 213 (1949).

⁵F. C. Larson, D. M. Coulson and F. C. Albright, *ibid.*, **196**, 45 (1952).

METHYL 4-HYDROXY-3,5-DIODOBENZOATE- I_2^{131}



J. H. Wilkinson, *Biochem., J.*, **54**, 485 (1953).

A. Procedure (Note 1)

In the following general method, the *p*-hydroxybenzoate (0.005 mole) is dissolved in 20–30 ml. of ethanol in a 100-ml. flask. A solution of 0.83 g. of potassium iodide and 0.1 mc. of sodium iodide- I^{131} in 5 ml. of water is added, followed by 7 ml. of 2 *N* acetic acid. The mixture is heated on a steam-bath under reflux, and a solution of 0.587 g. of iodic acid in 5 ml. of water is added gradually, through the condenser, over a period of 15 minutes. The mixture is heated until the iodine is completely ab-

sorbed, usually 30-60 minutes. An additional 0.83 g. of potassium iodide is then added, and heating is continued 90 minutes longer (Note 2). Water is added to induce crystallization of the product, which is collected after the mixture has cooled in an ice-bath for 2 hours. The yield of crude product is about 90% (Note 3). The ester is washed free of iodide and recrystallized from aqueous methanol or ethanol.

4-Hydroxy-3,5-diiodobenzoate- I_2^{131} esters, prepared by the above method, are given in the following table.

TABLE XV, 5

Ester	Recrystallization Solvent	m.p., °C.	Yield, %	Ref.
(a) Methyl	methanol	165	90	1
(b) Ethyl	ethanol	124	80-95	1
(c) Propyl	benzene	123	80-95	1
(d) Butyl	90% methanol	90	80-95	1
(e) Amyl	benzene-petroleum ether	78 (b.p., 60-80)	80-95	1
(f) Hexyl	petroleum ether	70-71 (b.p., 80-100)	80-95	1
(g) Heptyl	90% methanol	68	80-95	1
(h) Octyl	90% methanol	65	80-95	1
(i) <i>sec</i> -Butyl	116	2
<i>sec</i> -Butyl	90% ethanol	111	3
(j) 2-Hydroxyethyl	ethanol	164-165	4
(k) 2-Hydroxypropyl	90% ethanol	160-161	80	4
(l) 3-Hydroxypropyl	benzene-ethanol	154-155	72	4
(m) 4-Hydroxybutyl	80% ethanol	144-145	75	4
(n) 5-Hydroxypentyl	80% ethanol	131	76	4
(o) 6-Hydroxyhexyl	benzene	85-86	78	4
(p) 2,3-Dihydroxypropyl	80% methanol	128-129	69	4

B. Notes

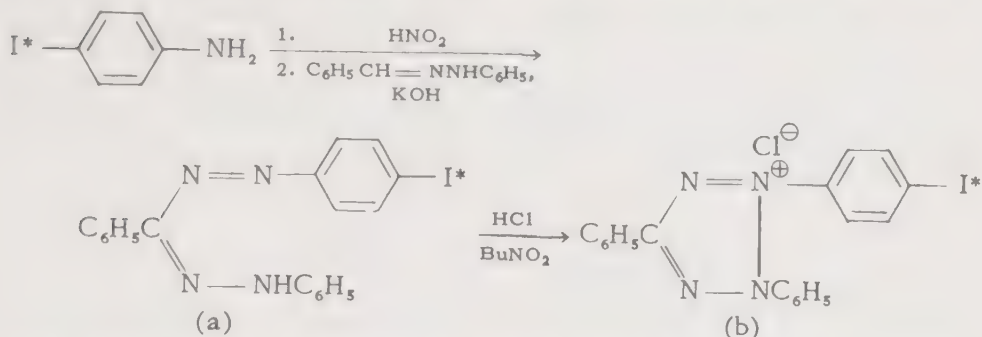
1. This is a general method applied to a number of esters.
2. By this time, the solution is almost colorless; if not, a little sodium thiosulfate is added to complete the decolorization.
3. The isotopic yield is 92-95%, allowance being made for decay of isotope.
4. The data in the table, obtained in nonisotopic preparations, are from the references cited.

¹M. M. Sheahan, J. H. Wilkinson and N. F. MacLagan, *Biochem. J.*, **48**, 188 (1951).

²J. H. Wilkinson, *ibid.*, **54**, 485 (1953).

³J. H. Wilkinson, M. M. Sheahan and N. F. MacLagan, *ibid.*, **49**, 712 (1951).

⁴J. H. Wilkinson, W. E. Sprott and N. F. MacLagan, *ibid.*, **54**, 16 (1953).

3-(4-iodophenyl)-2,5-diphenyl-2H-tetrazolium- I^{131} chloride


S. P. Masouredis, M. B. Shimkin, J. A. McMillan and S. W. Fox, J. Natl. Cancer Inst., 11, 91 (1950).

A. Procedure

(a) α -(4-Iodophenylazo)- α -phenylhydrazonotoluene- I^{131} . The formazan compound is prepared according to the following procedure of Fox and Atkinson.¹

4-Iodoaniline, 21.9 g. (0.1 mole), and 20 ml. of water are warmed together until the amine melts. Concentrated hydrochloric acid, 30 ml., is added slowly with vigorous shaking, and the mixture is cooled rapidly with stirring to effect precipitation of 4-iodoaniline hydrochloride in fine crystals. The suspension of amine hydrochloride, cooled to 0–5°, is diazotized with a cold solution of 7 g. of sodium nitrite in 15 ml. of water (Note 1).

The diazotized 4-iodoaniline and a solution of 19.6 g. of benzaldehyde phenylhydrazone in 1 l. of ethanol are added simultaneously, with vigorous stirring, to a solution of potassium hydroxide in 100 ml. of ethanol. The temperature is maintained at 20–30° (Note 2). Stirring is continued for 1 hour, and the mixture is set aside for at least 2 hours, and then filtered. The formazan, washed with 200 ml. of water followed by 100 ml. of alcohol and dissolved in hot dioxane (Note 3), is reprecipitated by adding slowly about 1/3 the volume of water with stirring. The product, dark red in color with a metallic green luster, m.p., 187–188°, is obtained in 45–60% yield.

(b) 3-(4-Iodophenyl)-2,5-diphenyl-2H-tetrazolium- I^{131} Chloride. In a mixture of 2.4 ml. of chloroform and 2.4 ml. of absolute methanol is dissolved 0.122 g. of α -(4-iodophenylazo)- α -phenylhydrazonotoluene- I^{131} . After 0.31 ml. of concentrated hydrochloric acid and 0.45 ml. of freshly distilled butyl nitrite are added in sequence, the solution is set aside for 90 minutes. The solution is concentrated, by boiling, to approximately 1 ml.; dioxane, 3 ml., is added, and the mixture is again concentrated to

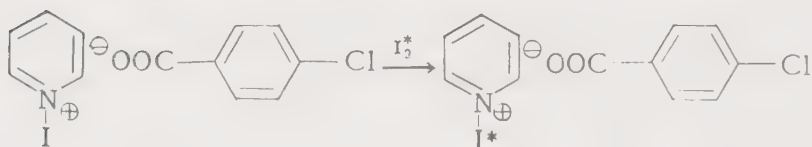
incipient crystallization. The mixture is centrifuged, and the solid, which is an impurity, is removed. To the supernatant liquid is added 3 ml. of dioxane, and the solution is again concentrated to crystallization. After centrifugation, the precipitate is washed with two 1.0-ml. portions of ether and dried in a vacuum desiccator. The yield is 33 mg., m.p. 229° (dec.) (cor.) (Note 4).

B. Notes

1. To a starch iodide end point.
2. A red color develops at once, and within a few minutes a red precipitate is observed.
3. About 20 ml. of boiling dioxane per g. of formazan.
4. The melting point observed approximates that of an analytically pure sample, $232-233^{\circ}$ (dec.).

¹S. W. Fox and E. H. Atkinson, J. Am. Chem. Soc., 72, 3630 (1950).

I-ODOPYRIDINIUM-I¹²⁸ 4-CHLOROBENZOATE



J. Kleinberg and J. Sattizahn, J. Am. Chem. Soc., 73, 1865 (1951).

A. Procedure (Note 1)

A weighed sample of isotopic iodine is made up to volume in pyridine to obtain the desired concentration. One ml. aliquots of this solution are introduced into ground-glass stoppered Erlenmeyer flasks, each of which contains 100 mg. of a unipositive iodine pyridine complex (Note 2) dissolved in 4 ml. of pyridine. After thorough mixing, some of the solutions are placed in a thermostat at 26.3° ; from others the complex is immediately thrown out of solution by treatment with petroleum ether. The complex is collected in a Hirsch funnel and washed with petroleum ether until the washings show no activity. The complex is air-dried and weighed. The thermostated solutions are withdrawn at appropriate intervals and treated in the same manner. The results of the exchange reactions are shown in the following table (Note 3). It is apparent from the data, in the following table, that elementary iodine exchanges completely with unipositive iodine complex immediately after mixing and

over a wide range of concentration ratios. Also, the exchange is independent of the nature of the organic anion in the complex.

TABLE XV, 6

Exchange between Isotopic Iodine and Pyridine-Coordinated Unipositive Iodine Complexes in Pyridine

Monopyridine iodine complex	Conc. of unipositive iodine (moles/l.)	Conc. of elementary iodine (moles/l.)	Exchange %
4-Chlorobenzoate	2.76×10^{-2}	1.04×10^{-1}	99.2
4-Chlorobenzoate	2.76×10^{-2}	3.44×10^{-7}	107.7
2-Naphthoate	2.66×10^{-2}	2.77×10^{-2}	102.0
3-Nitrobenzoate	2.69×10^{-2}	2.77×10^{-2}	100.9

B. Notes

1. It is generally assumed that the iodine in violet solutions exists primarily as iodine molecules; whereas, in brown solutions, the iodine is bound chemically in some manner to the solvent.^{1,2} The exact nature of the bond in the latter case has not been determined. Conductivity studies on iodine-pyridine solutions³ have been interpreted largely on the basis of an assumed dissociation of dissolved iodine into positive I-pyridine⁺ and negative I₃⁻ ions. A recent investigation⁴ of the absorption spectra of pyridine solutions of iodine and positive iodine salts of the type I-pyridine-X (where X = NO₃ or OOCR) adds support to this assumption. The exchange in pyridine solution between radioactive iodine and pyridine-coordinated unipositive iodine complexes of the general formula I-pyridine-OOCR is in accord with the ionization mechanism. The exchange is rapid and complete.

2. 1-Iodopyridinium 2-naphthoate, 4-chlorobenzoate and 3-nitrobenzoate were prepared by the reaction of inactive iodine with the silver salts of the appropriate acids in the presence of pyridine. Pyridine was dried for at least two weeks over sodium hydroxide pellets and then distilled just before use.

3. The exchange data in the table were determined immediately after mixing the radioactive iodine and unipositive iodine complex solutions. The values obtained for longer periods of time checked those which are given.

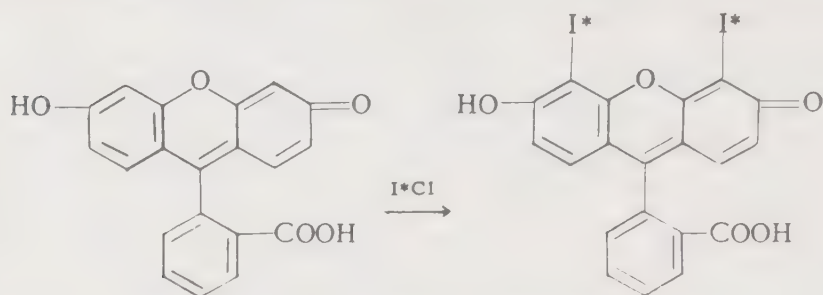
¹J. Kleinberg and A. W. Davidson, *Chem. Rev.*, **42**, 601 (1948).

²H. A. Benesi and J. H. Hildebrand, *J. Am. Chem. Soc.*, **71**, 2703 (1949); *ibid.*, **72**, 2273 (1950).

³L. F. Andrieth and E. J. Birr, *ibid.*, **55**, 668 (1933).

⁴R. A. Zingaro, C. A. VanderWerf and J. Kleinberg, *ibid.*, **73**, 88 (1951).

4,5-DIODOFLUORESC EIN- I_2^{131}
[9-(2-Carboxyphenyl)-6-hydroxy-4,5-diiodo-3H-isoxanth en-3-one- I_2^{131}]



G. Boyack, G. E. Moore and D. F. Clausen, *Nucleonics*, 3, 62 (1948).

A. Procedure

In a 250-ml. glass-stoppered flask, 0.76 g. (0.0031 mole), of dichloramine-T is dissolved in 15–20 ml. of glacial acetic acid, and 0.52 g. (0.0031 mole) of potassium iodide is added. The flask is shaken until the iodide dissolves and the orange-yellow color of iodine chloride appears. To this solution is added potassium iodide- I^{131} (10–15 mc.) in 4–5 ml. of water. Finally, 0.56 g. (1.65 mmoles) of fluorescein is added, and the flask is swirled to mix the contents and is then heated for 1 hour on the steam-bath. All of the fluorescein dissolves, producing a clear amber solution. This solution is poured into 225 ml. of water, and the precipitate of 4,5-diiodofluorescein- I_2^{131} is collected and dried in a sintered glass Büchner funnel, using vacuum. In a typical synthesis of 4,5-diiodofluorescein- I_2^{131} (Note 1) the yield is 81.6% (Note 2). However, Roe, Hayes and Bruner¹ (Note 3) have shown that this product very probably contains chlorine as well as iodine.

B. Notes

1. The location of the iodine in the molecule is assumed by analogy to the formation of 4,5-dibromofluorescein.²

2. In a similar experiment, a sodium bisulfite trap was placed in the vacuum line used in filtering; 94.8% of the iodine-131 was found in the finished product, 0.6% in the trap and 2.4% in the filtrate.

3. When Roe¹ attempted to adapt the iodinating procedure of Boyack³ to the preparation of 3,5-diiodo-4(1H)-pyridone- I_2^{131} , the product contained chlorine as well as iodine. It was necessary to decrease greatly the excess of dichloramine-T in order to obtain the desired product chlorine-free. Diiodofluorescein was then prepared according to the procedure of Boyack, Moore and Clausen³ and was found to contain 7.78% chlorine and 36.1% iodine; calculated for $C_{20}H_{10}O_5$, I_2 , I, 43.46%. It is noteworthy that

the total determined halogen content indicates some tri- and tetrahalogenation. Roe¹ also reported that it was not possible to prepare pure diiodofluorescein- I_2^{131} by their modification of the method for the pyridone synthesis.

C. Other Preparations

A simple synthesis of 4,5-diiodofluorescein- I_2^{131} is given by Vigne and Fondarai.⁴ Stoichiometric quantities of crystallized basic fluorescein and a 0.5% solution of iodine- I_2^{131} in chloroform are stirred together for 20 minutes.⁵ After removal of chloroform, ethyl acetate is added, the mixture is acidified, and the 4,5-diiodofluorescein- I_2^{131} dissolves in the solvent.

¹A. Roe, R. L. Hayes, and H. D. Bruner, J. Elisha Mitchell, Sci. Soc., 66, 163 (1950).

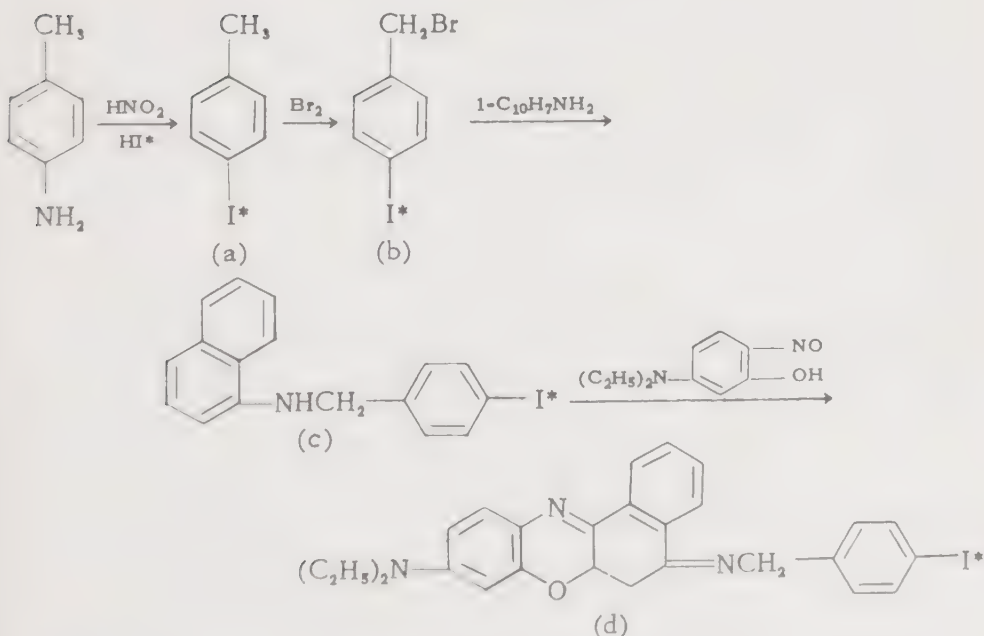
²R. B. Sandin, A. Gillies and S. C. Lynn, J. Am. Chem. Soc., 61, 1919 (1939).

³G. Boyack, G. E. Moore and D. F. Clausen, Nucleonics, 3, 62 (1948).

⁴J. Vigne and J. Fondarai, Nucleonics, 11, No. 9, 68 (1953).

⁵J. Fondarai and J. Vigne, Bull. soc. chim. France, 1953, 23; 1953, 331.

9-DIETHYLAMINO-5-(4-IODOBENZYLIMINO)-5H-BENZO[a]PHENOXAZINE- I^{131} (I^{131} -Nile Blue A)



H. A. Sloviter, Science, 110, 687 (1949).

A. Procedure

(a) *4-Iodotoluene- I^{131}* . A solution of 3.3 g. of *p*-toluidine in 35 ml. of water and 3.6 ml. of concentrated sulfuric acid is diazotized at 5° by the addition of 2.1 g. of sodium nitrite in 20 ml. of water. To 18.5 mc. of carrier-free iodide- I^{131} in 15 ml. of water is added 5 g. of potassium iodide, and this solution is slowly added to the cold diazonium salt (Note 1). The mixture is left in an ice-bath for 30 minutes, at room temperature for 1 hour, and is then heated gently on a steam-bath for 1 hour. The 4-iodotoluene- I^{131} is isolated from the mixture by steam distillation (Note 2). The distillate is made alkaline with sodium hydroxide, a small amount of sodium sulfite is added, and the 4-iodotoluene- I^{131} is extracted into 150 ml. of carbon tetrachloride. The extract is washed 3 times with water and evaporated to a volume of about 50 ml. (Note 3).

(b) *α -Bromo-4-iodotoluene- I^{131}* . To the solution of 4-iodotoluene- I^{131} in carbon tetrachloride is added 1.55 ml. of bromine in 25 ml. of carbon tetrachloride, and the flask is attached to an all-glass reflux condenser. The flask is illuminated by two 200-watt, clear, Mazda lamps, and the solution is gently refluxed for 3 hours, at which time the evolution of hydrogen bromide has ceased. The mixture is then cooled to room temperature, and 5 g. of potassium iodide in 25 ml. of water is added. Sodium thiosulfate solution is added until all the iodine is reduced. The carbon tetrachloride layer is separated and washed with water, and the carbon tetrachloride is distilled on a steam-bath.

(c) *N-(4-Iodobenzyl)-1-naphthylamine- I^{131}* . The residue of *α -bromo-4-iodotoluene- I^{131}* is dissolved in 50 ml. of ethanol, and a solution of 12 g. of 1-naphthylamine in 50 ml. of ethanol is added. The mixture is refluxed for 2 hours. The ethanol is distilled off, and the residue is extracted 3 times with 100-ml. portions of 1:50 hydrochloric acid to remove excess 1-naphthylamine. The product contains a small amount of 1-naphthylamine.

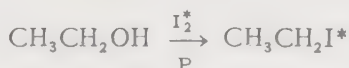
(d) *9-Diethylamino-5-(4-iodobenzylimino)-5H-benzo[a]phenoxazine- I^{131}* . The *N*-(4-iodobenzyl)-1-naphthylamine- I^{131} is dissolved in 75 ml. of ethanol and 9 ml. of concentrated hydrochloric acid. To this solution is added 10 g. of 5-diethylamino-2-nitrosophenol, dissolved in 25 ml. of ethanol. The mixture is gently boiled under reflux for 3 hours and then kept at room temperature overnight. After cooling the mixture in an ice-bath for 1 hour, the product is collected, washed with cold ethanol and then with ether. The yield of product is 8 g.

B. Notes

1. A small quantity of sodium sulfite is added to reduce any iodine.
2. A total distillate of 250 ml. is collected.

3. The intermediates were not isolated in this synthesis, although they easily could be.

IDOETHANE-I²⁸



P. Sue and J. Beydon, *Bull. soc. chim. France*, 11, 55 (1944).

A. Procedure (Note 1)

Isotopic sodium iodide is oxidized with sodium iodate¹ in dilute hydrochloric acid to obtain free iodine-I¹²⁸. The isotopic iodine is collected on a cellophane foil by centrifugation. The synthesis of ethyl iodide-I¹²⁸ is carried out in a 2-ml. flask with a side arm for introduction of the iodine on the cellophane and the phosphorus. Alcohol is added through an internal tube with stopcock. A cooled vertical condenser permits heating of the mixture of 10 parts of iodine, 10 to 20 parts of alcohol and 1 part of phosphorus under reflux; then by stopping the circulation of water, the product is distilled and condensed by a second, inclined condenser. The iodine color disappears after 5-10 minutes of heating on a water-bath. The distillation is rapid, and a small residual amount of ethyl iodide-I¹²⁸ is entrained by the distillation of two 1-ml. portions of alcohol (Note 2). Assay of the mixture of alcohol and ethyl iodide-I¹²⁸ by decomposition of the ethyl iodide-I¹²⁸ to silver iodide-I¹²⁸ indicates a yield of 28% (Note 3).

B. Notes

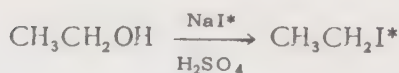
1. The ethyl iodide-I¹²⁸ (25-minute half-life) was prepared by a modification of the usual reaction of iodine and ethyl alcohol in the presence of phosphorus; the short half-life does not permit the use of long contact time.

2. Isolation of the ethyl iodide could be accomplished easily by high-vacuum distillation of the crude product through anhydrous calcium chloride and phosphorus pentoxide.

3. Comparison of the activity of the sodium iodide used and the silver iodide, obtained from decomposition of the synthesized ethyl iodide, indicated a yield of 20%.

¹P. Sue, *Compt. rend.*, 212, 237 (1941).

iodoethane- I^{131}

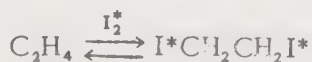


H. Ludes and P. Endler, *Z. ges. inn. Med.*, 7, 423 (1952); through *Chem. Abstracts*, 46, 9145 (1952).

Procedure

To 2.6 ml. of 95% ethanol is added 2.6 ml. of concentrated sulfuric acid, and the mixture is cooled to room temperature. To this mixture are added 1.8 ml. of a sodium iodide- I^{131} solution and 3 g. of powdered sodium iodide. After 10 minutes, the ethyl iodide- I^{131} is distilled into ice-water, and free iodine is removed by the addition of a few drops of sodium bisulfite solution. The yield of ethyl iodide- I^{131} is 46.4%.

1,2-diiodoethane- I_2^{131}



A. Abrams and T. W. Davis, *J. Am. Chem. Soc.*, 76, 5993 (1954).

A. Procedure (Note 1)

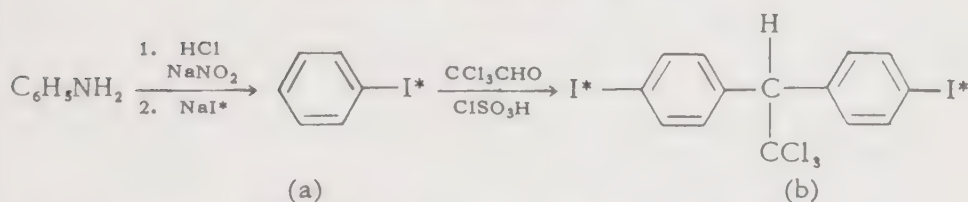
An ampoule containing iodine- I_2^{131} is nicked with a file and placed in a reaction vessel of the desired capacity. The vessel is evacuated, and ethylene is admitted and condensed with liquid nitrogen while the reaction vessel is sealed with a flame (Note 2). After allowing the reaction vessel to warm up, the ampoule is broken by shaking the vessel. After a period of 12 hours or more at temperatures above 25° , the reaction vessel is cooled in liquid nitrogen and opened, and 20 ml. of carbon tetrachloride is added. The reaction mixture is then warmed to 0° , and the solution of iodine- I_2^{131} and 1,2-diiodoethane- I_2^{131} is acidified with acetic acid and titrated with 0.005 N thiosulfate until the free iodine is consumed. The two layers are separated, and the aqueous phase is extracted with two portions of carbon tetrachloride (Note 3).

B. Notes

1. This procedure was designed to give data on the equilibrium between 1,2-diiodoethane and its dissociation products: ethylene and iodine.
2. Ethylene was introduced to a pressure judged sufficient to transform half the iodine to diiodoethane at equilibrium.

3. The two solutions containing iodide- I^{131} ion and 1,2-diiodoethane- I_2^{131} , respectively, were each diluted to 100 ml. and counted separately. The 1,2-diiodoethane- I_2^{131} should be obtainable through evaporation of the carbon tetrachloride solution.

1,1,1-TRICHLORO-2,2-BIS(4-IODOPHENYL)ETHANE- I_2^{131}
(Iodine-131 analog of DDT)



J. A. Jensen and G. W. Pearce, J. Am. Chem. Soc., 74, 2436 (1952).

A. Procedure

(a) *Iodobenzene- I^{131}* . To 10 ml. of distilled water and a cube of ice in a 250-ml. beaker are added 2 ml. (0.022 mole) of freshly distilled aniline and 9.5 ml. of concentrated hydrochloric acid. The mixture is stirred until the temperature drops to about -10° . Then a solution of 1.52 g. (0.022 mole) of sodium nitrite in 8 ml. of water is added slowly by pipet, keeping the tip immersed while the mixture is rapidly stirred (Note 1). When the nitrite is in slight excess (Note 2), the solution is transferred to a 150-ml. round-bottomed flask (Note 3) and cooled to -5° . Approximately half of a solution of inactive sodium iodide, 3.28 g. (0.022 mole) in 10 ml. of water, is added to the flask, and the contents are mixed (Note 4). Then, 25 mc. of sodium iodide- I^{131} in 2.3 ml. of solution is added with gentle mixing, and the remainder of the inert sodium iodide is added also. With an air condenser attached to the reaction flask, it is removed from the cold bath, and the reaction mixture warms to room temperature. After standing for 6 hours, the contents of the flask are heated to about 60° for 1 hour. Gentle swirling of the reaction mixture aids in settling the iodobenzene- I^{131} to the bottom of the flask, and most of the supernatant liquid is removed by decantation. To the residue of iodobenzene- I^{131} is added 10 ml. of 15% sodium hydroxide and several sodium hydroxide pellets. From the strongly alkaline solution, the iodobenzene is steam-distilled immediately. A 70% yield (1.7 ml., 0.015 mole) of crude iodobenzene- I^{131} is obtained.

(b) *1,1,1-Trichloro-2,2-bis(4-iodophenyl)ethane- I_2^{131}* . The 1.7 ml. of crude iodobenzene- I^{131} and 0.7 ml. of inactive iodobenzene are transferred to a special reaction flask (Note 5), and 0.8 ml. of freshly distilled

chloral is added. The flask is immersed in an ice-salt bath at -5 to 0° , and with vigorous stirring of the reactants, 1.4 ml. (0.021 mole) of chlorosulfonic acid (Note 6) is added in 0.1-ml. amounts, every 5–10 minutes. After addition of the chlorosulfonic acid, the mixture is stirred for an additional 6 hours keeping the temperature between -5 and 0° . The mixture is a thick slurry at this time, and the reaction is quenched by addition of ice and ice-water. The mixture is drained into a flask containing 10 ml. of 2% sodium carbonate solution. The crude product is washed several times, by decantation, with warm water, and is then collected on a fritted-glass filter. After drying on the filter with vacuum, the crude product is dissolved in hot acetone and transferred to a cone-shaped flask fitted with a stopcock at the apex, a standard taper joint at the top and a side arm for applying vacuum. The solvent is evaporated in a stream of air with the aid of an infrared lamp. Last traces of solvent and any unreacted iodobenzene- I^{131} or chloral are removed by heating the evacuated flask at 50 – 60° for 3 hours (Note 7).

The crude product, 2.35 g., is dissolved in 55 ml. of 1:1 ethanol-acetone solution heated under reflux. The flask is then cooled in an ice-bath for 4 hours to crystallize the product. The white, crystalline product is collected on a fritted-glass filter and washed three times with 10-ml. portions of ethanol cooled with Dry Ice. After drying at 100° , the product weighs 1.42 g. (23.5% based on sodium iodide- I^{131}) and melts at 173 – 174° (Note 8).

B. Notes

1. Addition of the nitrite is controlled such that the temperature does not rise above -5° .
2. Excess nitrite is detected with starch-iodide paper.
3. The flask has been rinsed with a solution of 0.02 mole of sodium hydroxide, 0.005 mole of sodium bisulfite, and 0.0015 mole of sodium iodide in 1000 ml. of water.
4. In initial trials, only 1 to 5% of the radioactivity was recovered in the final product. This is overcome by addition of the active sodium iodide to the benzenediazonium chloride solution after 50–70% of the inert sodium iodide has been added.
5. The special flask is a separatory funnel equipped with a stirrer and addition tube.
6. No really satisfactory condensing agent was found, but chlorosulfonic acid gave the best yields (55–60% based on chloral).
7. A small amount of radioactive material, probably unreacted iodobenzene, was recovered in a Dry Ice-acetone trap inserted in the vacuum line.
8. As in the case of DDT, the crude product was a mixture of *o,p*- and *p,p'*-iodophenyl isomers. Since only the *p,p'*-isomer was desired for

experimental use, various recrystallization solvents were tested. The 1 to 1 ethanol-acetone solvent was found to give a product of high purity with a single recrystallization. The *p,p'*-isomer is obtained as indicated by the melting point. The filtrate contains largely the *o,p*-isomer of lower melting point.

C. Other Preparations

Keneshea and Kahn¹ have reported the preparation of high specific activity iodobenzene- I^{131} , in about 40% yield, by the irradiation of solutions of benzene-chlorobenzene containing tellurium tetrachloride in the thermal column of a Los Alamos reactor.

Iodobenzene- I^{131} has also been prepared:² in 51% yield, b.p. 126.2–126.7° (138 mm.), $n_D^{17.9}$ 1.6200, from sodium iodide- I^{131} and aniline by the method of Gatterman and Wieland;³ in 57% and 71% yields by the reaction of potassium iodide- I^{131} with benzenediazonium chloride;⁴ and in 66% yield from benzene, iodine- I_2^{131} and nitric acid.⁵

¹F. J. Keneshea and M. Kahn, J. Am. Chem. Soc., 74, 5254 (1952).

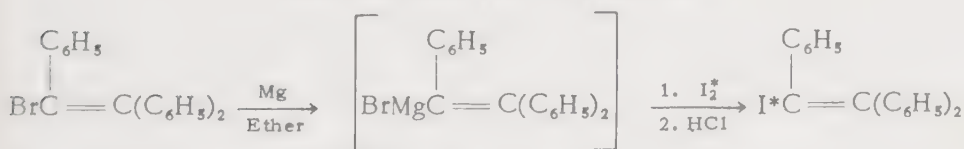
²J. D. Roberts, J. K. Sanfor, F. L. J. Sixma, H. Cerfontain and R. Zagt, J. Am. Chem. Soc., 76, 4525 (1954).

³L. Gatterman and H. Wieland, *Laboratory Methods of Organic Chemistry*, The MacMillan Co., New York, N. Y., 1938, p. 281.

⁴*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 351.

⁵*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 323.

IODOTRIPHENYLETHYLENE- I^{131}



D. C. Morrison, J. Am. Chem. Soc., 74, 4459 (1952).

A. Procedure

In an atmosphere of nitrogen 1 g. of magnesium is treated with 0.3 ml. of ethyl bromide in 25 ml. of ether (Note 1). After the reaction is well started, 1 g. of bromotriphenylethylene¹ (m.p. 114°) is added in several portions during 10–15 minutes, and the mixture is heated under reflux for 2.5 hours. The grey solution (Note 2) is cooled and treated with a benzene solution of iodine- I_2^{131} . Solid, inactive iodine is then added until its color in the solution is permanent (Note 3); then the mixture is hydrolyzed with a mixture of ice and hydrochloric acid.

The ether-benzene layer is washed with bisulfite solution and with water and is then concentrated to dryness. The residue in ether-petroleum ether solution is decolorized with carbon, and the solvent is again

evaporated. The crystalline residue is extracted with four small portions of cold petroleum ether by grinding under this solvent; this removes a small amount of oil. The iodo compound is recrystallized from petroleum ether or from alcohol. One recrystallization from petroleum ether gives a product of m.p. 125.5–127°; Koelsch¹ gives 126–127°. The yield of iodotriphenylethylene-I¹³¹ is approximately 68% (Note 4).

B. Notes

1. The ethyl bromide serves only to start the reaction. The amount specified is considerably more than that used by Koelsch,¹ who also added a single crystal of iodine.

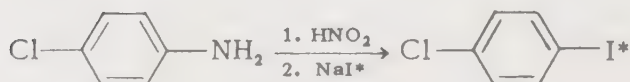
2. The solution is yellow if air has been admitted.

3. So that maximum utilization of the isotope will be made, an insufficient amount of the carrier iodine is used, and the last of the Grignard reagent is then reacted with the inactive iodine.

4. This was determined in preliminary runs of about the same magnitude.

¹C. F. Koelsch, J. Am. Chem. Soc., 54, 2046 (1932).

1-CHLORO-4-IODOBENZENE-I¹³¹



D. Atack and W. G. Schneider, J. Phys. and Colloid Chem., 54, 1323 (1950).

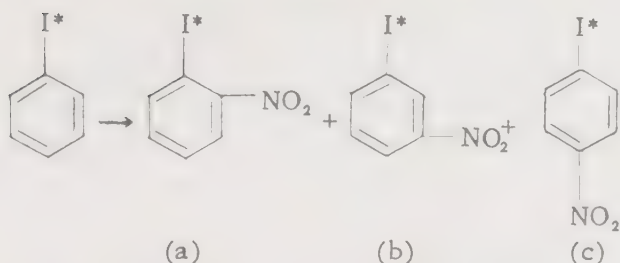
Procedure

4-Chloroaniline is diazotized¹ at 0–5° in approximately 6 N hydrochloric acid by the addition of a concentrated solution of sodium nitrite. When a slight excess of nitrous acid is present (starch iodide paper), the solution is stirred for 10 minutes. A solution of sodium iodide-I¹³¹ with potassium iodide carrier is then added, and the solution is kept at room temperature for several hours and is then heated under reflux on a steam-bath until no more gas is evolved^{1,2} (see 4-iodotoluene-I¹³¹). The product is isolated by steam distillation and purified by sublimation under reduced pressure. A white crystalline solid, m.p. 55°, is obtained. The product may also be crystallized from ethanol, m.p. 56°.³

¹Organic Syntheses, Coll. Vol. II, Wiley, New York, 1943, p. 353.

²H. A. Sloviter, Science, 110, 687 (1949).

³R. L. Datta and N. R. Chatterjee, J. Am. Chem. Soc., 41, 292 (1919).

1-iodo-2-nitrobenzene- I^{131} 

J. D. Roberts, J. K. Sanford, F. L. J. Sixma, H. Cerfontain and R. Zagt, J. Am. Chem. Soc., 76, 4525 (1954).

A. Procedure

Nitration of iodobenzene- I^{131} . To a mixture of 8.409 g. (0.412 mole) of iodobenzene- I^{131} and 20 ml. of nitromethane at 25° , in a 250-ml. glass-stoppered flask, is added gradually, with stirring, a mixture of 2.60 ml. (0.062 mole) of anhydrous nitric acid and 3.72 ml. (0.039 mole) of acetic anhydride (Note 1). After 52 hours at 25° , the flask is filled with ice, shaken and set aside for 12–15 hours. The products of nitration are then isolated by continuous extraction with carbon disulfide. The carbon disulfide extract is diluted to 250 ml. and divided into three fractions (Note 2).

(a) *1-iodo-4-nitrobenzene- I^{131} .* Fraction B, 200 ml., is mixed with 5 ml. of 0.3463 *N* iodine in carbon disulfide, set aside for one-half hour and extracted with sodium thiosulfate solution (Note 3). The carbon disulfide solution is concentrated, and most of the *p*-isomer crystallizes. After it is twice recrystallized from acetone-alcohol solution, containing inactive *o*- (1.0 g.) and *m*-isomers (0.5 g.) and 1 ml. of inactive iodobenzene, the 1-iodo-4-nitrobenzene- I^{131} melts at 174.6 – 175.4° (Note 4).

(b) *1-iodo-3-nitrobenzene- I^{131} .* The *m*-isomer was obtained by distillation of the residual carbon disulfide solution to remove the solvent and iodobenzene- I^{131} . The crude product is heated under reflux with 50 ml. of piperidine for 10 hours; then, the reaction mixture is acidified and steam-distilled. The crude product is sublimed and again heated under reflux for 2 days with piperidine, containing 2 g. each of the inactive *o*- and *p*-isomers. The *m*-isomer, isolated as above, is dissolved in ether, washed with sodium hydroxide and hydrochloric acid solutions, recovered by evaporation of the solvent and recrystallized from pentane containing 1 ml. of inactive iodobenzene; m.p. 37.8 – 38.5° (Note 5).

(c) *1-iodo-2-nitrobenzene- I^{131} .* Fraction C is neutralized and concentrated until the *p*-isomer crystallizes. The carbon disulfide solution

containing the *o*-isomer is then twice scavenged with 5-g. portions of *p*-isomer and 1-g. portions of *m*-isomer. Unreacted iodobenzene- I^{131} is removed by distilling successively three 2-ml. portions of inactive iodobenzene from the *o*-isomer, which is finally recrystallized from pentane. Three fractions were obtained with melting points respectively of 50.4–51.0°, 50.7–51.7°, and 50.9–51.6° (Note 6).

B. Notes

1. The acid and anhydride are mixed at 0°, see 1-chloro-2-nitrobenzene- Cl^{36} .

2. Fraction A, 5 ml. (2%), was used for determination of the total activity of the extracted products. Fraction B, 200.0 ml. (80%), was used for the determination of 1-iodo-3- and -4-nitrobenzene- I^{131} , iodobenzene- I^{131} , and iodine- I_2^{131} , and was diluted with 9.8106 g. of 1-iodo-3-nitrobenzene, 10.3014 g. of 1-iodo-4-nitrobenzene and 9.9512 g. of iodobenzene. Fraction C, 25.0 ml. (10%), was used for the determination of the *o*-, *p*-isomer ratio and was mixed with 19.7386 g. of 1-iodo-2-nitrobenzene and 9.9295 g. of 1-iodo-4-nitrobenzene. Fractions B and C were each diluted with additional solvent and refluxed until homogenous solutions resulted.

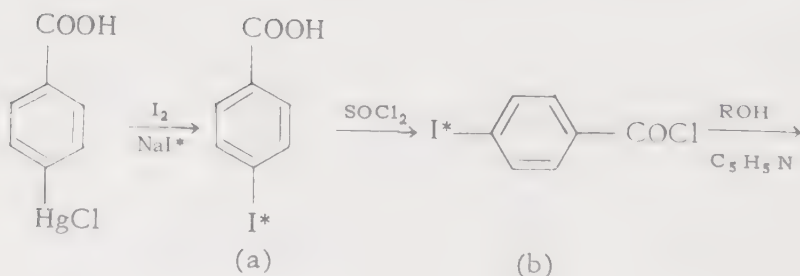
3. For removal of iodide and iodine formed during the nitration.

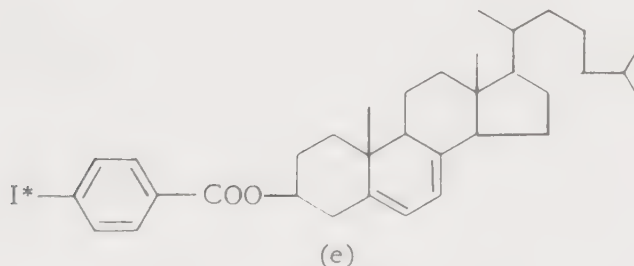
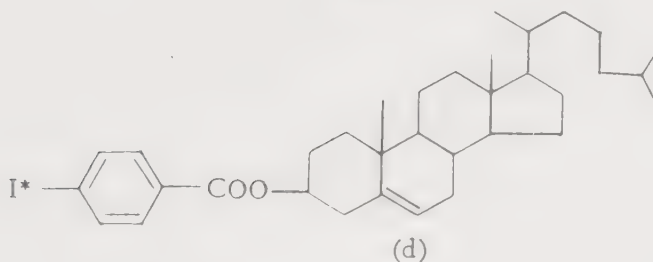
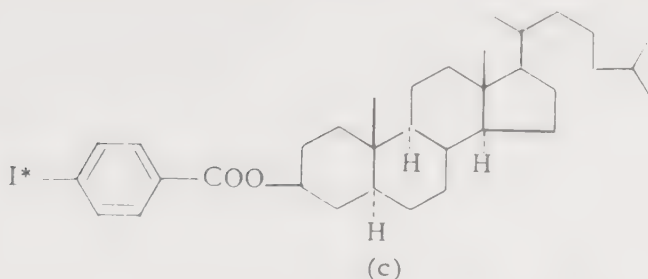
4. The activity did not change significantly during the second recrystallization.

5. Additional scavenging with inactive *o*- and *p*-isomers did not change the activity significantly.

6. The distribution of isomers found in the nitration of iodobenzene- I^{131} was: *o*-, 38.3%; *m*-, 1.8%; *p*-, 59.7%.

5,7-CHOLESTADIEN-3 β -YL 4-IODOBENZOATE- I^{131} (7-Dehydrocholesteryl 4-Iodobenzoate- I^{131})





W. M. Stokes, F. C. Hickey, O. P. Fish and W. A. Fish, J. Am. Chem. Soc., 76, 5174 (1954).

A. Procedure

(a) *4-Iodobenzoic- I^{131} Acid* (Note 1). To a solution of 1.5 g. (0.0059 mole) of iodine in 25 ml. of 95% alcohol is added 5 mc. of sodium iodide- I^{131} , in approximately 2 ml. of water, and 3.0 g. (0.0084 mole) of powdered, dry 4-chloromercuribenzoic acid.¹ As the mixture is stirred and heated under reflux, the acid gradually goes into solution, and the color of iodine disappears. The hot, stirred mixture is then treated with iodine until a yellow color persists for at least 10 minutes (Note 2). Any insoluble material is filtered from the hot solution, and the filtrate is cooled to obtain the crystalline product, which is carefully dried in a vacuum oven at 60°.

(b) *4-Iodobenzoyl- I^{131} Chloride*. The dry 4-iodobenzoic- I^{131} acid is refluxed for 2 hours with 10 ml. of thionyl chloride. The excess thionyl chloride is removed under vacuum, and the residue of 4-iodobenzoyl- I^{131} chloride is purified by vacuum sublimation; m.p. 65°.

(c) *3 β -Cholestanyl 4-iodobenzoate- I^{131}* . To a solution of 100 mg. (0.25 mmole) of 3 β -cholestanol in dry pyridine is added 0.55 mmole of 4-iodobenzoyl- I^{131} chloride. The solution is kept at 5° for 12 hours. The yield of crude ester is 90–96%. After recrystallization, the product melts at 186° to a cloudy melt clearing at 230°.

(d) *Cholesteryl 4-iodobenzoate- I^{131}* . This ester, m.p. 186° to a cloudy melt clearing at 230°, is prepared from cholesterol in 90–96% yield according to the above procedure.

(e) *5,7-Cholestadien-3 β -yl 4-iodobenzoate- I^{131}* . Also prepared in the above manner from 7-dehydrocholesterol, the product gives a cloudy melt at 178.5°, with decomposition.

B. Notes

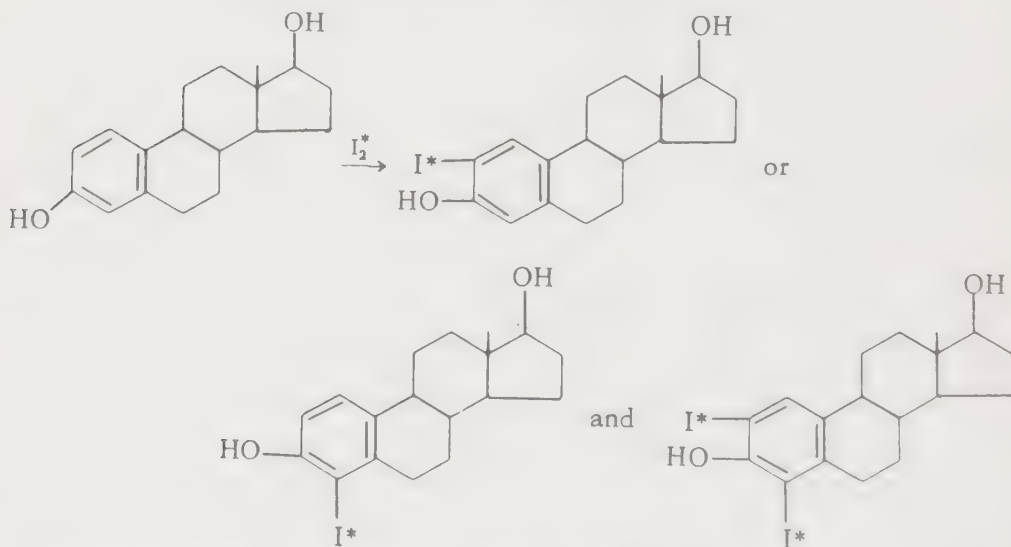
1. The synthesis of Whitmore and Woodward² was employed at 1/100 the original scale.

2. It is convenient to add a solution of iodine in alcohol.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941 p. 159.

²*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 325.

2,4-DIIODOESTRADIOL- I^{131}



S. Albert, R. D. H. Heard, C. P. Leblond and J. Saffran, *J. Biol. Chem.*, 177, 247 (1949).

A. Procedure (Note 1)

The iodine isotope, present as iodide, in 1 ml. of 1% sodium hydroxide solution is mixed with 0.1032 mg. of carrier potassium iodide and 0.0266

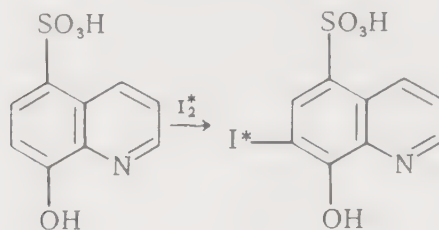
mg. of potassium iodate and acidified with 0.2 ml. of 2.5 *N* sulfuric acid to release iodine- I_2^{131} . The estradiol, 0.100 mg. dissolved in 1 ml. of methanol and 0.2 ml. of concentrated ammonium hydroxide, is added. The reaction mixture, pH 9 to 10, is neutralized with glacial acetic acid, diluted to 10 volumes with distilled water, and extracted with three 15-ml. portions of freshly distilled ether. The combined ether extracts are twice washed with water and evaporated. The resulting product is an oil which is comprised of estradiol, I^{131} -monoiodoestradiol and I_2^{131} -diiodoestradiol (Note 2). In order to obtain 2,4-diiodoestradiol- I_2^{131} , the material from a preparation with iodine- I_2^{131} is dissolved in benzene with 4 mg. of unlabeled, crystalline 2,4-diiodoestradiol. About 2.7 mg. of 2,4-diiodoestradiol- I_2^{131} slowly crystallizes from the solution.

B. Notes

1. Model experiments for the iodination of estradiol were performed with either iodine in alkaline aqueous medium or *N*-iodoacetamide under anhydrous conditions. From both methods a yellow oil was obtained which was purified chromatographically on alumina and crystallized from benzene and petroleum ether. The melting point of this microcrystalline material (173–174°, dec.) was depressed on admixture with pure estradiol (m.p. 176–177°). These crystals contained 0.480 mg. of iodine per mg. and were thus diiodoestradiol.

2. The composition of the product was determined by application of isotope dilution techniques and chromatographic separations to a similar active preparation which was diluted with a large amount of nonradioactive iodinated estradiol. After the product was mixed with water, the organic material was dissolved in ether and chromatographed on alumina. Three crystalline compounds and several oily fractions were obtained. From 340.6 mg. of oily mixture, 38.5 mg. of diiodoestradiol, m.p. 173–174.5° (dec.), was obtained by elution with benzene containing 1% acetone. The second crystalline fraction, eluted with benzene containing 4–8% acetone, was 48.2 mg. of unreacted estradiol, m.p. 176–177°. The third crystalline fraction, m.p. 168–169.5° (dec.), was monoiodoestradiol, as was the major part of each of the subsequent fractions.

8-HYDROXY-7-iodo-5-QUINOLINESULFONIC- I^{131} ACID
(Chiniofon- I^{131})

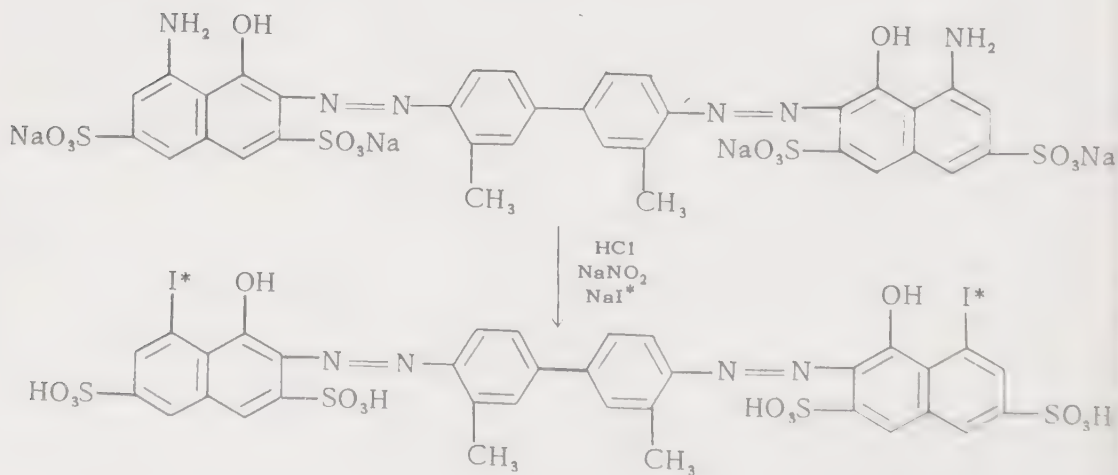


E. C. Albright, D. L. Tabern and E. S. Gordon, *Am. J. Trop. Med.*, 27, 553(1947).

Procedure

To a concentrated solution of calcium iodide- I_2^{131} (prepared from active iodide and 9.0 g. of potassium iodate) is added 8.6 g. of 8-hydroxy-5-quinolinesulfonic acid. The solution is heated to boiling, and 3.6 g. of calcium hypochlorite is added. The solution is cooled and, with stirring, dilute hydrochloric acid is added, during several hours, at such a rate that an excess of liberated iodine is avoided. The iodinated product is purified by dissolution in sodium carbonate solution from which it is precipitated by acidification with hydrochloric acid. The chiniofon- I^{131} is collected, washed with alcohol and dried; the yield is 10-11 g.

2,2'-(3,3'-DIMETHYL-4,4'-BIPHENYLENEBISAZO)BIS(8-iodo-1-NAPHTHOL-3,6-DISULFONIC- I^{131} ACID)
(I_2^{131} -Iodotrypan Blue)



H. S. Bloch and F. E. Ray, *J. Natl. Cancer Inst.*, 7, 64 (1946).

A. Procedure

Tetrasodium Trypan Blue, 320 mg. (0.33 mmole), is dissolved in 5 ml. of water, and the dark blue solution is acidified with 1 ml. of 5 M sulfuric acid (5 mmoles). The amino groups are then diazotized at 0-5°, by the gradual addition of 46 mg. (0.66 mmole) of sodium nitrite dissolved in 1 ml. of water (Note 1). The diazotization mixture is stirred for 0.5 hour at 0-5°; then 100 mg. of ammonium sulfamate is added to destroy any excess nitrite, and stirring is continued for another 0.25 hour. Then 1 ml. of isotopic iodide solution, of the desired activity, containing 15 mg. (0.1 mmole) of ordinary sodium iodide is added. The solution is slowly warmed to 50° and stirred at that temperature for 6 hours. The color changes to purple-red, and finally the solution is heated to 96-100° and kept at that temperature for another hour. After the solution is cooled to room temperature, the dye is precipitated by the addition of 50 ml. of ethyl alcohol and 100 ml. of peroxide-free ethyl ether and separated from the mother liquor by centrifugation. The I_2^{131} -Iodotrypan Blue, after drying, contains approximately 70% of the radioactivity initially introduced (Note 2).

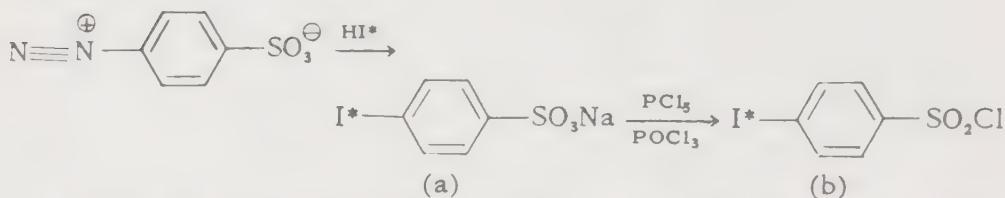
B. Notes

1. The color of the solution changes to green-grey.

2. According to Bloch and Ray, the final product is a mixture of hydroxy and I_2^{131} -Iodotrypan Blue. Stevens¹ used the preparative procedure of Bloch and Ray, and after reprecipitating the crude dye four times from water with 1:2 ethanol-ether, it was mixed with 50 ml. of ethanol saturated with ammonia. After 1 hour, the product was again precipitated with ether, washed with ethanol-ether and dried. The dye, after an initial heating at 80° and storage over Drierite, lost 5 to 6% of its weight on further heating at 80 to 90°. This product had the following approximate composition as determined from elementary analyses: 52% dihydroxy Trypan Blue, 40% monoiodo monohydroxy Trypan Blue and 3% diiodo Trypan Blue.

¹C. D. Stevens, A. Lee, P. H. Stewart, P. M. Quinlin and P. R. Gilson, *Cancer Research*, 9, 139 (1949).

4-IODOBENZENESULFONYL-I¹³¹ CHLORIDE
(Pipsyl-I¹³¹ Chloride)



A. S. Keston, S. Udenfriend and R. K. Cannon, *J. Am. Chem. Soc.*, **71**, 249 (1949).

A. Procedure

(a) *Sodium 4-iodobenzenesulfonate-I¹³¹*. To a solution of the iodine-131 isotope, in the form of iodide ion (Note 1), is added sufficient potassium iodide to make a total of 25 mg. The solution is adjusted to a pH above 7, and evaporated to a volume of about 0.2 ml., and a few small crystals of sodium sulfite are added. After cooling the solution, an equal volume of concentrated hydrochloric acid is added, followed by 25 mg. of 4-diazobenzenesulfonic acid (Note 2). After the initial evolution of nitrogen, the mixture is warmed to complete the reaction; the reaction mixture is again cooled, and another 15 mg. each of 4-diazobenzenesulfonic acid and potassium iodide are added and allowed to react as before. The solution is then saturated with sodium chloride and cooled; the sodium salt of 4-iodobenzenesulfonic-I¹³¹ acid crystallizes out. The crystals are collected by centrifugation and washed with brine. The combined mother liquor and washings are warmed, and about 40 mg. of nonisotopic sodium 4-iodobenzenesulfonate, in a small volume of water, is added. The added sodium salt crystallizes from the cold solution carrying part of the residual isotopic analog. This is isolated and washed as before, and the procedure is repeated.

(b) *Pipsyl-I¹³¹ Chloride*. The separate batches of sodium salt are dried, each is dissolved in about 1 ml. of phosphorus oxychloride containing an excess of phosphorus pentachloride, and the solutions are combined. The solution is heated gently to assure reaction and transferred to a separatory funnel containing ice water and about 50 ml. of benzene. Small portions of benzene are used to facilitate the transfer. At this time, approximately 200 mg. of pure nonisotopic pipsyl chloride is added. The benzene layer is washed several times with cold water to remove excess phosphorus halides (Note 3). The benzene solution is dried over anhydrous sodium sulfate, and the drying agent is washed with benzene (Note 4).

The combined benzene solution is evaporated to a small volume and transferred to a cold finger micro-distillation apparatus. The benzene is evaporated in a stream of air, and the pipsyl chloride is distilled onto

the cold finger at 150° and a few mm. pressure. The distilled material is washed into a flask with a minimum of benzene (Note 5). The material is crystallized by adding petroleum ether to the benzene and concentrating the resulting solution. The small amount of solvent is removed with a capillary pipet, and the crystals are dried at 70° for 1 hour, m.p. 86° (Note 6).

B. Notes

1. An irradiation unit containing approximately 100 mc. of iodine-131 was used for each isotopic synthesis. The iodine-131 was separated from the tellurium according to the procedure developed by Levy, Keston and Undenfriend.¹

2. 4-Diazobenzenesulfonic acid is prepared² in quantities of about 100 g. each and treated with potassium iodide, while still wet, to obtain 4-iodobenzenesulfonic acid.³

3. The rate of hydrolysis of pipsyl chloride is negligible under these conditions.

4. The amount of washing necessary is evident from the activity remaining in the drying agent.

5. At this point more nonisotopic pipsyl chloride may be added to make a desired specific activity.

6. The literature⁴ gives 86–87°.

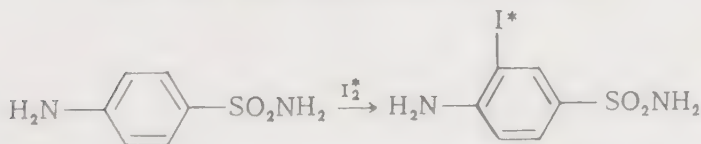
¹M. Levy, A. S. Keston and S. Undenfriend, J. Am. Chem. Soc., 70, 2289 (1948).

²C. Weygand, *Organic Preparations*, Interscience Publishers, Inc., New York, 1945, p. 109.

³C. Weygand, *ibid*, p. 112.

⁴W. Lenz, Ber., 10, 1136 (1877).

3-IODOSULFANILAMIDE-I¹³¹ (4-Amino-3-iodobenzenesulfonamide-I¹³¹)



H. S. Bloch and F. E. Ray, J. Natl. Cancer Inst., 7, 65 (1946).

A. Procedure

To 8.5 g. of sulfanilamide (0.05 mole) is added 15 ml. of concentrated hydrochloric acid and then 500 ml. of water. On stirring at room temperature, all the sulfonamide dissolves, and the solution is cooled to 10° by the addition of ice. To the cooled solution, 25 ml. of sodium

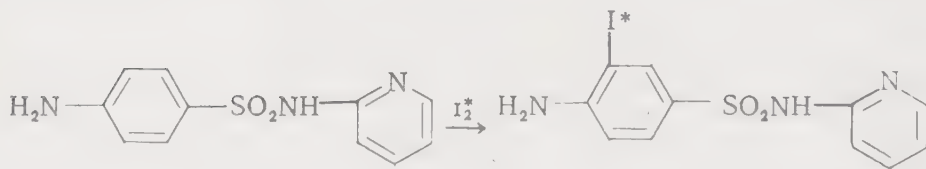
iodide- I^{131} solution is added, and then, with effective stirring, 8 g. of iodine monochloride (0.05 mole). A beige-colored precipitate appears. The mixture is stirred at room temperature for 1 hour and is then set aside for 2 days. The beige-brown precipitate, which settles from the clear, slightly yellow supernatant liquid, is collected on a filter, washed and dried *in vacuo* over phosphorus pentoxide. The yield of beige-colored, amorphous 3-iodosulfanilamide- I^{131} is 11 g. (74%).

The crude 3-iodosulfanilamide- I^{131} is dissolved, at the boiling point, in 600 ml. of water, which contains 10.4 g. of sodium bisulfite (0.1 mole), and is acidified with 2 ml. of 6 *N* hydrochloric acid. The hot, yellow solution is filtered quickly with vacuum (Note 1), and the filtrate is cooled at about 3° for 3 hours. The white, crystalline precipitate is collected on a filter, washed with water (Note 2) and dried *in vacuo* over phosphorus pentoxide. The yield of white, crystalline 3-iodosulfanilamide- I^{131} is 7 g. (47%) (Note 3).

B. Notes

1. There is no residue.
2. The product is washed with water until the washings no longer decolorize a dilute aqueous iodine solution.
3. The radioactivity yield in the recrystallized product is 58%.

3-iodo-*N*¹-2-pyridylsulfanilamide- I^{131} (Iodosulfapyridine- I^{131})



H. S. Bloch and F. E. Ray, *J. Natl. Cancer Inst.*, 7, 65 (1946).

A. Procedure

Sulfapyridine hydrate, 28.9 g. (0.1 mole), is dissolved in 1000 ml. of water containing 35 ml. of concentrated hydrochloric acid. To the solution, cooled to 10° by adding ice, is added 50 ml. of sodium iodide- I^{131} solution, and then, with effective stirring, 16 g. of iodine monochloride (0.1 mole). The mixture is stirred at room temperature for 1 hour. The beige-colored precipitate is collected (Note 1), washed with water and dried over phosphorus pentoxide. The yield of crude (Note 2) iodosulfapyridine- I^{131} is 38 g. (100%).

The crude compound, 34 g., is suspended in 1000 ml. of water, containing 21 g. (0.2 mole) of sodium bisulfite and 10 ml. of 6 *N* hydrochloric acid, which is heated to boiling. The solid remains largely undissolved, and the mixture is cooled in an ice-bath. The solid is collected on a filter and washed with water (Note 3) and finally with ether. The iodosulfapyridine- I^{131} , dried over phosphrous pentoxide *in vacuo*, weighs 31g. (91%) and is light ochre in color and amorphous (Note 4).

B. Notes

1. After a while, a small amount of white precipitate separates from the greenish-yellow filtrate.
2. The crude product is dark beige and amorphous.
3. The product is washed with water until the washings no longer decolorize a dilute aqueous iodine solution.
4. The isotopic yield in the final product is 64%.

HYDROGEN IODIDE- I^{131}



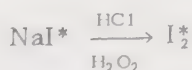
R. D. Heyding and C. A. Winkler, *Can. J. Chem.*, 29, 790 (1951).

Procedure

Aqueous sodium iodide- I^{131} is evaporated to dryness in a storage trap attached to a vacuum line. Then, inactive hydrogen iodide is prepared by dropwise addition of water to 20 parts of iodine and 1 of red phosphorus. The gas evolved is purified by passing it through red phosphorus and two cold traps, the first at 0° and the second at -35° . The hydrogen iodide, m.p. -51° , is collected in the storage trap cooled with acetone-Dry Ice and allowed to liquify and exchange with the sodium iodide- I^{131} . The exchange is essentially complete in 2 hours at -45° , and the isotopic hydrogen iodide is distilled into a second cold trap.

IODINE- I_2^{131}

METHOD I



J. C. Clayton, A. A. Free, J. E. Page, G. F. Somers and E. A. Woollett, *Biochem. J.*, 46, 598 (1950).

A. Procedure

To a solution of sodium iodide- I^{131} (1 mc.) in approximately 1 ml. of buffered bisulfite solution, pH 7, is added 2.3 mg. of inactive sodium iodide. The solution, in a centrifuge tube, is covered with 1 ml. of ether, and 1 drop of 1 *N* hydrochloric acid and excess 0.3% hydrogen peroxide solution (approximately 0.5 ml.) are added. After occasional mixing during 2 hours, the lower iodine-free layer is removed by means of a pipet and washed with 1 ml. of ether. The combined ether layers contain the iodine- I_2^{131} .

METHOD II



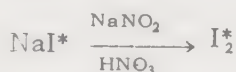
R. M. Lemmon, W. Tarpey and K. G. Scott, *J. Am. Chem. Soc.*, 72, 758 (1950).

A. Procedure

Carrier-free sodium iodide- I^{131} , 0.028 μ g. (3050 μ c.), in 1.0 ml. of a sodium bisulfite buffered solution is added gradually to 238 μ g. (143 μ moles) of potassium iodide in 30 μ l. of water. By means of a heat lamp and a stream of nitrogen blown over the surface, the solution is concentrated to approximately 10 μ l.

To the iodide solution cooled in an ice-bath, 120 μ g. (0.56 μ mole) of potassium iodate in 20 μ l. of water and 11 μ l. of glacial acetic acid are added, giving 1.72 μ equivalents of iodine- I_2^{131} .

METHOD III



D. C. Morrison, *J. Am. Chem. Soc.*, 74, 4459 (1952).

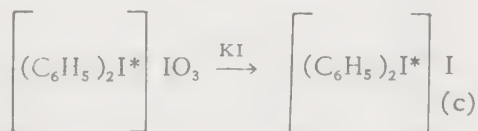
A. Procedure

Carrier sodium iodide, 0.3 g., is dissolved in water in a separatory funnel, and the desired amount of sodium iodide- I^{131} is added. The aqueous solution is covered with an equal volume of benzene, and then 0.4 g. of sodium nitrite in a concentrated aqueous solution is added. With shaking, the mixture is treated dropwise with 6 *N* nitric acid until an excess is present. If necessary, more acid is added until the aqueous phase remains colorless. After 20 minutes, the layers are separated, and the organic layer is washed once by extraction with water. The benzene solution of iodine- I_2^{131} is then ready for use, after drying over sodium sulfate, if necessary.

Free¹ also used the peroxide method of oxidation. Albright² prepared active iodine by the action of iodate on the isotopic iodide, then reduced the iodine to iodide with sulfur dioxide and converted it to calcium iodide. Iodine-I₂¹³¹ was again liberated in an iodination reaction mixture by addition of dilute hydrochloric acid to a solution of the calcium iodide-I₂¹³¹ and calcium hypochlorite. In a number of instances³⁻⁷ free iodine-I₂¹³¹ has been prepared by the oxidation of iodide with iodate in the presence of either sulfuric or hydrochloric acid. The nitrite method of oxidation has been used also by Seligman,⁸ who extracted the free iodine-I₂¹³¹ into carbon tetrachloride. Free iodine-I₂¹³¹ has also been prepared⁹ by the addition of iodine monochloride to sodium iodide-I¹³¹ solution.

⁹H. S. Bloch and F. E. Ray, *J. Natl. Cancer Inst.*, **7**, 65 (1946).

(b)



A. Procedure

(b) *Iodosobenzene-I¹³¹*. Iodobenzene-I¹³¹ dichloride is hydrolyzed with sodium hydroxide according to the procedure of Lucas, Kennedy and Formo.² The yield of iodosobenzene-I¹³¹ is 60-62%.

(c) *Diphenyliodonium-I¹³¹ Iodide*. Equivalent amounts of iodosobenzene-I¹³¹, 5 g., and iodoxybenzene, 5.3 g., are stirred vigorously for 4-5 hours in 500 ml. of water with the theoretical quantity of freshly precipitated silver hydroxide. The silver iodate is filtered off, and the diphenyliodonium-I¹³¹ iodide is precipitated by the addition of potassium iodide. It is then collected on a filter and washed with water, ethanol and finally ether (Note 1). According to Juliusburger³ (Note 2) there is no exchange between the two iodine atoms.

B. Notes

1. Although the yield of radioactive product was not given, according to Lucas and Kennedy,⁴ the yield is 70-72% of diphenyliodonium iodide melting at 172-175° with vigorous decomposition.

2. Juliusburger,³ has prepared diphenyliodonium iodide-I¹³¹ by exchange. The inactive iodide is crystallized from a hot aqueous-alcoholic solution containing sodium iodide-I¹³¹. The solid, after washing with ice-cold water, is strongly radioactive. The activity is shown to be present as the negative iodide ion. The iodide ion is removed with silver, the diphenyliodonium hydroxide solution is filtered, and the iodide is again precipitated by the addition of inactive sodium iodide. The resultant precipitate is also inactive; thus the exchange occurs with the negative iodide ion only. Another experiment demonstrated that no interchange occurs between the two iodine atoms.

¹*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 69.

²*Ibid.*, p. 70.

³F. Juliusburger, B. Topley, and J. Weiss, *J. Chem. Soc.*, 1935, 1295.

⁴*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 52.

I₂¹³¹-DIODODEUTEROPORPHYRIN DIMETHYL ESTER

A. Treibs, *Naturwissenschaften*, 39, 281 (1952).

Deuteroporphyrin dimethyl ester is heated for a long period in a sealed tube with iodine-I₂¹³¹, pyridine and chloroform. I₂¹³¹-Diiododeuteroporphyrin dimethyl ester forms quantitatively, m.p. 239°. The product has a hydrochloride number of 14, forms metal complexes in the normal manner, and is saponified with either 20% hydrochloric acid or potassium hydroxide in pyridine-methanol. The potassium salt is very soluble in water.

I¹³¹-BOVINE SERUM ALBUMIN

W. C. Knox and F. C. Endicott, *J. Immunol.*, 65, 523 (1950).

A. Procedure

Sufficient potassium iodide and potassium iodate are added to a slightly alkaline solution of carrier-free iodide- I^{131} to liberate, when acidified, one mg. of elemental iodine per 100 mg. of protein to be iodinated. The theoretical amount of 0.2 *N* sulfuric acid is added, and the free iodine usually precipitates as a colloidal or fine suspension. A few drops of 0.1 *M* disodium acid phosphate are added, and the iodine suspension is then added dropwise to the cold, agitated solution of bovine serum albumin in 0.1 *M* disodium acid phosphate (Note 1). After all the iodine is added, the reaction mixture is kept overnight in the cold.

After the volume is reduced, dialysis is carried out against repeated changes of distilled water. When the protein is removed from the sack, an aliquot is taken, to which is added an excess of previously prepared antibody to bovine serum albumin. If less than 95% of the radioactivity of the aliquot is retained in the twice-washed specific precipitate, dialysis is repeated. From 4 to 8% (8 to 16% of theoretical) yields are obtained on the basis of radioiodine (Note 2).

B. Notes

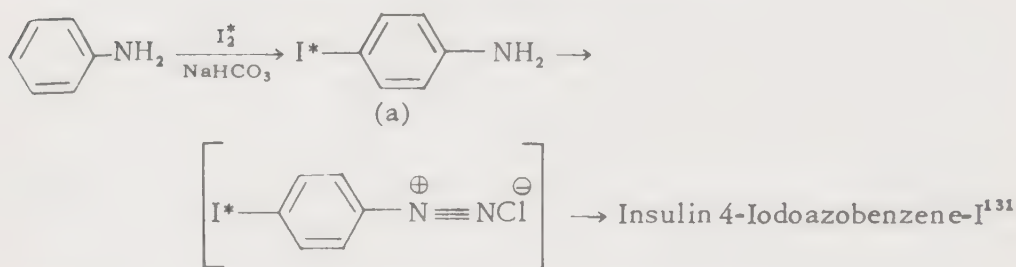
1. A few reactions, carried out in a Na_2HPO_4 - NaH_2PO_4 buffer at pH 6.8, gave erratic yields.
2. Each 100 mg. of protein contained from 0.04 to 0.08 mg. of iodine, including carrier, or less than one atom of iodine per molecule of protein.

C. Other Preparations

All of the procedures described are similar. Warren and Dixon¹ iodinated bovine gamma globulin in 25-30% yield in the presence of sodium carbonate. Fine and Seligman² iodinated bovine albumin in sodium carbonate solution; 15% of the iodine was incorporated in the protein. Bovine albumin was also iodinated by Eisen and Keston³ in a buffered medium at pH 8 to 9; 2 to 4% of the initial radioactivity was bound in the protein. Pressman and Eisen⁴ iodinated rabbit serum globulin and reported that 10 to 20% of the initial iodine combined with the protein. Pressman⁵ iodinated globulin, and about 10% of the iodine combined with the protein; this is about 20% of the amount that could have combined. Masouredis,⁶ iodinated globulin, also, in phosphate buffer medium. Purification by the usual dialysis procedure gave a globulin preparation containing 10% of the iodine activity. All but 1.6% of the activity was retained in the protein fraction precipitated by chloracetic acid. Frances⁷ has described procedure for the introduction of varying amounts of isotopic iodine into proteins; the reactions were run in ammoniacal solution.

- ¹S. Warren and F. J. Dixon, *Am. J. Med. Sci.*, **216**, 136 (1948).
²J. Fine and A. M. Seligman, *J. Clin. Invest.*, **23**, 721 (1944).
³H. N. Eisen and A. S. Keston, *J. Immunol.*, **63**, 72 (1949).
⁴D. Pressman and H. N. Eisen, *J. Immunol.*, **64**, 274 (1950).
⁵D. Pressman, *Cancer*, **2**, 698 (1949).
⁶S. P. Masouredis, L. R. Melcher and D. C. Kobleck, *J. Immunol.*, **66**, 297 (1951).
⁷G. E. Frances, W. Mulligan and A. Wormall, *Nature*, **167**, 748 (1951).

INSULIN 4-iodoazobenzene-I¹³¹



L. Reiner, A. S. Keston and M. Green, *Science*, **96**, 362 (1942).

A. Procedure (Note 1)

(a) *4-Iodoaniline-I¹³¹*. A solution of sodium iodide-I¹³¹, containing less than 30 micrograms of iodine, is evaporated to a volume of about 0.05 ml. The iodine-I¹³¹ is liberated by acidification with hydrochloric acid and the addition of an excess of potassium iodate, both the acid and the iodate being contained in about 0.01 ml. of solution. The solution is made alkaline with sodium bicarbonate, a slight excess of aniline is added, and the mixture is stirred intermittently for 0.5 hour. Since the sodium iodide-I¹³¹ which is formed in this reaction must contain at least half of the isotope, iodine-I¹³¹ is again liberated by addition of potassium iodate and acid, using about half the amounts of reagents used before. By repeating this procedure a third time, more than half of the isotopic iodine is converted to iodoaniline-I¹³¹.

(b) *Insulin 4-Iodoazobenzene-I¹³¹*. The above mixture is acidified with hydrochloric acid and cooled to 0°, and the 4-iodoaniline-I¹³¹ is diazotized by the addition of sodium nitrite. Five mg. of insulin dissolved in the minimum amount of 0.1 N hydrochloric acid is added, and the pH is adjusted to 8-9 for coupling the diazonium salt. The solution is set aside for at least one-half hour. The product is then precipitated from solution three times at its isoelectric point in the presence of ordinary potassium iodide, 4-iodophenol and 4-iodoaniline.

B. Notes

1. This procedure is according to the micro technique of Morton.¹ A very similar procedure was employed by Reiner² using a larger sample of radioactive iodine.

¹A. A. Morton, *Laboratory Techniques in Organic Chemistry*, McGraw-Hill, New York, 1938.

²L. Reiner, E. H. Lang, J. W. Irvine, Jr., W. Peacock and R. D. Evans, *J. Pharmacol. Exptl. Therap.*, 78, 352 (1943).

I¹³¹-INSULIN

W. C. Stadie, N. Haugaard and M. Vaughn, *J. Biol. Chem.*, 199, 731 (1952).

A. Procedure

To 8 to 10 mc. of I₂¹³¹ contained in a test tube is added 2 drops of a solution which is 0.07 M in ferrous sulfate and 0.05 M in ferric sulfate in 1 M sulfuric acid, and the mixture is set aside for 5 minutes. To this mixture is then added 5 mg. of insulin dissolved in about 5 ml. of M/15 phosphate buffer, pH 7.4, and the pH of the mixture is adjusted to 7.4 with dilute sodium hydroxide. The mixture is stirred intermittently for 1.5 to 2 hours (Note 1). After dialysis in a cellophane sack for 72 hours against running water and finally against a fixed volume of distilled water, the protein content and radioactivity of the insulin solution are determined (Note 2).

B. Notes

1. The iodination has been done both at room temperature and at 0° without apparent difference in the result.

2. Preparations containing from 5.6 to 8.9×10^6 c.p.m. per mg. are obtained with no demonstrable inactivation of the insulin as measured by its effect on glycogen synthesis in the isolated rat diaphragm.

I¹³¹-POLYSACCHARIDE

A. M. Seligman, M. J. Shear, J. Leiter and B. Sweet, *J. Natl. Cancer Inst.*, 9, 13 (1948).

A. Procedure

To 1 ml. of a solution containing 2.5 mg. of polysaccharide is added 0.1 ml. of 10% sodium carbonate (10 mg.), followed by 0.5 ml. of a carbon tetrachloride solution of iodine-I₂¹³¹, 0.25 mg. (Note 1). The mixture is

shaken immediately with rapid decolorization of the organic phase. The aqueous layer is diluted to 5 ml., separated and dialyzed through cellophane tubing in running tap water for 72 hours (Note 2).

B. Notes

1. The iodination may also be done without the addition of sodium carbonate.

2. Radioactivity measurements indicated that 3.5% of the iodine is incorporated into the polysaccharide.

I^{131} -SOYA BEAN OIL

A. M. Rutenburg, A. M. Seligman and J. Fine, J. Clin. Invest., 28, 1105 (1949).

A. Procedure

(a) *Iodine- I^{131} Monochloride*. Potassium iodide, 3.6 g., is dissolved in 6.2 ml. of distilled water, to which one to two equivalents of carrier-free iodide- I^{131} and 2.4 g. of potassium iodate are added. Concentrated hydrochloric acid, 7.2 ml., is added slowly with vigorous stirring, and the resulting heavy, dark precipitate of iodine- I^{131} monochloride slowly redissolves (Note 1). The final solution is clear and orange-colored (Note 2).

(b) *I^{131} -Soya Bean Oil*. A modified vegetable oil iodination method¹ (Note 3) is used for iodination of soya bean oil (Note 4) with iodine- I^{131} monochloride. The iodine- I^{131} monochloride solution is cooled to 4–5° and added in three portions, with stirring, to a cold solution of 50–55 g. of soya bean oil in 100 ml. of ether. The mixture is mechanically shaken for 60–90 minutes at room temperature; during this time, the color of the aqueous solution is discharged. The straw-yellow oil-ether solution is washed once with water and twice with 5% sodium sulfite in 0.1 N sodium hydroxide solution. It is then washed with water, dilute hydrochloric acid, and finally several times with distilled water (Note 5). The ether solution is dried with anhydrous sodium sulfate, and the ether is removed by distillation under reduced pressure and at a temperature between 40–50°. The yield of residual, pale-yellow oil is 56 g. (Note 6).

B. Notes

1. A small amount of iodine vapor appears in the flask during the procedure.

2. A highly diluted aliquot is taken for radioactivity measurement.

3. By this method only one atom of iodine is incorporated upon halogenation of an ethylenic linkage. By using an excess of the oil an average of 0.46 atom of iodine is incorporated into each molecule of fat.

4. The refined oil used contained 33.7% oleic acid, 52.0% linoleic acid, 2.3% linolenic acid and 11.2% palmitic and stearic acids. Molecular weight of this oil is about 876, and each average molecule contains approximately 4.3 double bonds.

5. The combined washings contained 2% of the total radioactivity used in the iodination.

6. No radioactivity due to inorganic iodine could be extracted from the oil with sodium sulfite solution after a sample stood in the dark for 10 days. In several such preparations, 80-90% of the I^{131} used was incorporated into the fat.

¹S. Kimura, *Tohoku J. Exper. Med.*, 30, 336 (1937).

TABLE XV, 7
Iodine Exchange with Alkyl Iodides

Formula	Compound	Conc., mol./l.	Isotope source	Conc., mol./l.	Time, min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
CHI ₃	Iodoform	NaI ¹²⁸	100	ethanol	Exchange takes place.	4
CHI ₃	Iodoform	~2	NaI ¹²⁸	~0.5	1	room	95% ethanol	10	2
CH ₂ I ₂	Diiodomethane	0.48	NaI ¹²⁸	0.06	60	ethanol	2nd order $k = 1.036 \pm 0.022 \times 10^{-3}$ l./mol. sec.	5
CH ₂ I ₂	Diiodomethane	NaI ¹²⁸	100	ethanol	Exchange takes place.	4
CH ₂ I ₂	Diiodomethane	~2	NaI ¹²⁸	~0.5	1	room	95% ethanol	<10	CH ₂ I ₂ * obtained by adding excess water.	2
CH ₃ I	Iodomethane	NaI ¹³¹	acetone	To isolate CH ₃ I*, the acetone was precipitated as bisulfite compound.	1
CH ₃ I	Iodomethane	~2	NaI ¹²⁸	~0.5	1	room	95% ethanol	>50	CH ₃ I* obtained by adding excess water.	2
CH ₃ I	Iodomethane	I ₂ ¹³¹	400	+	Detection of free radicals; vapor phase.	3
CH ₃ I	Iodomethane	NaI ¹²⁸	15	room	ethanol	100	Exchange also in acetone and amyl alcohol.	4
CH ₃ I	Iodomethane	I ₂ ¹²⁸	15	100	ethanol	~100	No reaction at room temp. in 2-3 min. No reaction at 100° without solvent	4

CH ₃ I	Iodomethane	NaI ¹²⁸	ethanol	100	Exchange is rapid.	5
C ₂ H ₅ I ₂	<i>trans</i> -1,2-Diiodo- ethylene	I ₂ ¹³¹	0.04	0.002	30	60	Illuminated with a tungsten lamp.	7
C ₃ H ₇ I ₂	<i>trans</i> -1,2-Diiodo- ethylene	I ₂ ¹³¹	0.012	0.0026	35	55	Illuminated with two 500 watt bulbs. Per cent of total act.	11
C ₂ H ₅ I ₂	<i>cis</i> -1,2-Diiodo- ethylene	I ₂ ¹³¹	0.012	0.0026	35	60	Illuminated with two 500 watt bulbs. Per cent of total act.	11
C ₂ H ₅ I ₂	<i>trans</i> -1,2-Diiodo- ethylene	I ₂ ¹³¹	0.0052	0.0026	99.8	45	Per cent of total activity.	11
C ₂ H ₅ I ₂	<i>cis</i> -1,2-Diiodo- ethylene	I ₂ ¹³¹	0.0052	0.0026	room	50	Per cent of total activity.	11
C ₂ H ₅ I	Iodoethylene	I ₂ ¹³¹	Detection of free radicals ob- tained by gamma irradiation of pentane.	6
C ₂ H ₅ IO ₂	Iodoacetic acid	NaI ¹³¹	room	4
C ₂ H ₄ I ₂	1,2-Diiodoethane	I ₂ ¹³¹	0.07	2.64 × 10 ⁻³	100	15	Thermal exchange.	12
C ₂ H ₄ I ₂	1,2-Diiodoethane	I ₂ ¹³¹	0.04	3.3 × 10 ⁻³	25	43	Light from incandescent lamp.	12
C ₂ H ₅ I	Iodoethane	~2 NaI ¹²⁸	~2	~0.5	room	<10	2
C ₂ H ₅ I	Iodoethane	I ₂ ¹³¹	From free radicals obtained by gamma irradiation of pentane.	6
C ₂ H ₅ I	Iodoethane	I ₂ ¹³¹	0.04	0.002	30	Illuminated with tungsten lamp, exchange significant.	7
C ₂ H ₅ I	Iodoethane	100	~100	Exchange is nearly complete after 15 min. at 50-55°.	4

(Continued)

TABLE XV, 7 (Continued)

Formula	Compound	Conc., mol./l.	Isotope source	Conc., mol./l.	Time, min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
C_2H_5I	Iodoethane	0.91	NaI^{128}	0.135	50	ethanol	2nd order $k = 3.2 \pm 0.18 \times 10^{-3}$ l./mol. sec.	5
C_3H_5I	Iodoethane	19.76×10^{-3}	NaI^{128}	17.76×10^{-3}	30	70.1	dry ethanol	38.8	Per cent of total activity in alkyl iodide. Rate $k = 2.03 \pm 0.05 \times 10^{-3}$ sec $^{-1}$.	8
C_2H_5I	Iodoethane	0.044	NaI^{131}	0.023	25	methanol	Rate $k = 8.0 \times 10^{-3}$.	9,10
C_3H_5I	3-Iodopropene	0.04	I_2^{131}	0.002	20 sec.	30	hexane	60	Per cent of equilibrium.	7
C_3H_5I	3-Iodopropene	2	NaI^{128}	0.5	1	room	95%	>50	2
$C_3H_5IO_2$	2-Iodopropionic acid	NaI^{128}	1-2	~100	water	~100	Does not exchange at room temperature.	4
C_3H_7I	1-Iodopropane	~2	NaI^{128}	~0.5	1	room	95% ethanol	<10	Isolated by adding excess water.	2
C_3H_7I	1-Iodopropane	I_2^{131}	400	+	Detection of free radicals.	3
C_3H_7I	1-Iodopropane	NaI^{128}	15	100	ethanol	~100	4
C_3H_7I	1-Iodopropane	0.75	NaI^{128}	0.135	55	ethanol	2nd order $k = 2.35 \times 10^{-3}$ l./mole sec.	5
C_3H_7I	1-Iodopropane	19.59×10^{-3}	NaI^{128}	14.48×10^{-3}	45	70.1	dry ethanol	39.7	Per cent of activity in alkyl iodide; $k = 13.6 \times 10^{-3}$ sec $^{-1}$.	8
C_3H_7I	1-Iodopropane	I^{131}	pentane	From free radicals obtained by gamma irradiation of pentane.	6
C_3H_7I	2-Iodopropane	~2	NaI^{128}	~0.5	1	room	95% ethanol	<10	Isolated by adding excess water.	2
C_3H_7I	2-Iodopropane	NaI^{128}	15	100	ethanol	~100	4

C_3H_7I	2-Iodopropane	1.62	NaI^{128}	0.142	70	ethanol	2nd order $k = 6.98 \times 10^{-3}$ l./mole sec.	5
C_3H_7I	2-Iodopropane	19.88×10^{-3}	NaI^{128}	18.44×10^{-3}	180	70.1	dry ethanol	15.7	Per cent of total act. in alkyl iodide $k = 9.6 \times 10^{-4}$ sec $^{-1}$.	8
$C_4H_{10}I$	1-Iodobutane	NaI^{128}	15	100	ethanol	~100	4
$C_4H_{10}I$	1-Iodobutane	0.64	NaI^{128}	0.135	60.5	ethanol	$k = 2.85 \times 10^{-3}$ l./mole sec.	5
$C_4H_{10}I$	1-Iodobutane	I_2^{31}	3.1×10^{-3}	pentane	From free radicals obtained by gamma irradiation of pentane.	6
$C_4H_{10}I$	2-Iodobutane	1.42	NaI^{128}	0.142	70	ethanol	$k = 8.82 \times 10^{-4}$ l./mole sec.	5
C_4H_9I	1-Iodo-2-methylpropane	1.42	NaI^{128}	0.142	70	ethanol	$k = 8.48 \times 10^{-4}$ l./mole sec.	5
C_4H_9I	2-Iodo-2-methylpropane	1.24	NaI^{128}	0.37	40	ethanol	$k = 9.4 \times 10^{-4}$ l./mole sec.	5
C_4H_9I	2-Iodo-2-methylpropane	~ 0.2	NaI^{128}	1.27×10^{-5}	0	liquid SO_2	$k = 1.6 \times 10^{-4}$ sec $^{-1}$.	13
$C_5H_{11}I$	1-Iodopentane	0.56	NaI^{128}	0.135	60.5	ethanol	$k = 3.56 \times 10^{-3}$ l./mole sec.	5
$C_5H_{11}I$	1-Iodopentane	I_2^{31}	3.1×10^{-3}	pentane	From free radicals obtained by gamma irradiation of pentane	6
$C_5H_{11}I$	2-Iodopentane	I_2^{31}	3.1×10^{-3}	pentane	From free radicals obtained by gamma irradiation of pentane.	6
$C_5H_{11}I$	1-Iodo-3-methylbutane	0.715	NaI^{128}	0.137	50	ethanol	$k = 5.72 \times 10^{-4}$ l./mole sec.	5

(Continued)

TABLE XV, 7 (Continued)

Formula	Compound	Conc., mol./l.	Isotope source	Conc., mol./l.	Time, min.	Temp., °C.	Solvent	% Ex- change	Notes	Ref.
C ₅ H ₁₁ I	1-Iodo-3-methyl- butane	...	NaI ¹²⁸	...	15	100	ethanol	~100	...	4
C ₇ H ₉ I	α-Iodotoluene	3.75 × 10 ⁻³	KI ¹³¹	3.95 × 10 ⁻³	23.3	44	methanol	50	k = 3.86 l./mole min.	14
C ₇ H ₉ I	α-Iodotoluene	2.48 × 10 ⁻³	KI ¹³¹	2.05 × 10 ⁻³	18.5	44	ethanol	50	k = 8.29 l./mole min.	14
C ₈ H ₁₇ I	2-Iodo-octane	0.1377	NaI ¹³¹	0.1600	...	30	acetone	...	k = 3.00 × 10 ⁻³ l./mole min. Study of Walden inversion.	15

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TABLE XV, 8
Iodine Exchange with Aryl Iodides

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time, min.	Temp., °C.	Solvent	% Ex. change	Notes	Ref.
$C_6H_5I_3O$	2,3,6-Triiodo-phenol	KI^{131}	50	50% methanol	+	3
$C_6H_4INO_2$	1-Iodo-2-nitrobenzene	0.147	NaI^{131}	0.037	238	aceto-nitrile	87.5	2nd order rate constant = 5.96 liter-mole ⁻¹ hr ⁻¹ .	1
$C_6H_4INO_2$	1-Iodo-3-nitrobenzene	0.368	NaI^{131}	0.037	238	aceto-nitrile	89	1st order rate constant = 30.4 × 10 ⁻⁴ hr ⁻¹ .	1
$C_6H_4INO_2$	1-Iodo-4-nitrobenzene	0.074	NaI^{131}	0.037	238	aceto-nitrile	66	2nd order rate constant = 1.78 liter-mole ⁻¹ hr ⁻¹ .	1
$C_6H_4I_2O$	2,6-Diiodo-phenol	KI^{131}	50	50% methanol	+	3
$C_6H_4I_2O$	4,6-Diiodo-phenol	KI^{131}	50	50% methanol	+	3
C_6H_5I	Iodobenzene	0.662	NaI^{131}	0.037	238	aceto-nitrile	88	1st order rate constant = 8.9 × 10 ⁻⁴ hr ⁻¹ .	1
C_6H_5I	Iodobenzene	0.04	I_2^{131}	0.002	30	hexane	Illuminated with a tungsten lamp.	2
$C_7H_6I_2O$	3,5-Diiodo- <i>p</i> -cresol	KI^{131}	50	50% methanol	+	3

(Continued)

TABLE XV, 8 (Continued)

Formula	Compound	Conc. mol./l.	Isotope source	Conc. mol./l.	Time, min.	Temp., °C.	Solvent	% Ex. change	Notes	Ref.
C ₉ H ₉ I ₂ NO ₃	3,5-Diiodo-tyrosine-I ₂ ¹³¹	2 × 10 ⁻³	1.20 × 10 ⁻³	15	25	water, pH 2	62	Per cent of equilibrium. Reverse exchange. Exchange rate increased at higher pH values.	3
C ₉ H ₉ I ₂ NO ₃	3,5-Diiodo-tyrosine	2.2 × 10 ⁻³	KI ¹³¹	2.2 × 10 ⁻⁴	3	90	water, pH 5	97	Maximum exchange rate at pH 4 to 5.5 at all temps.	3
C ₁₂ H ₁₀ I ₂	Diphenyliodonium iodide	NaI ¹²⁸	ethanol or water-ethanol	+	Only the negative iodine exchanged.	4
C ₁₅ H ₁₁ I ₄ NO ₄	L-Thyroxine	I ₂ ¹³¹	50% ethanol	10-50	Medium at pH 4 to 5.	5,6
C ₁₅ H ₁₁ I ₄ NO ₄	Thyroxine	2.57 × 10 ⁻⁴	I ₂ ¹³¹	3.15 × 10 ⁻²	720	b.p.	90% butanol	30	Medium at pH 5.	7

¹A. M. Kristjanson and C. A. Winkler, *Can. J. Chem.*, **29**, 154 (1951).²R. M. Noyes, *J. Am. Chem. Soc.*, **70**, 2614 (1948).³W. H. Miller, G. W. Anderson, R. K. Madison and D. J. Salley, *Science*, **100**, 340 (1944).⁴F. Juliusburger, B. Topley and J. Weiss, *J. Chem. Soc.*, 1295, 1295.⁵A. Taurog, F. N. Briggs and I. L. Chaikoff, *J. Biol. Chem.*, **194**, 655 (1952).⁶*Ibidem*, **191**, 29 (1951).⁷E. Frieden, M. B. Lipsett and R. J. Winzler, *Science*, **107**, 353 (1948).

ISOTOPIC HYDROGEN COMPOUNDS

A. DEUTERIUM COMPOUNDS

FORMIC-H² ACID

R. C. Herman and V. Z. Williams, J. Chem. Phys., 8, 447 (1940).

A. Procedure

(a) *Oxalic Acid-H₂²*. Anhydrous oxalic acid is repeatedly treated with water-H₂² and dehydrated by heating under vacuum after the final exchange.

(b) *Formic-H² Acid-H²*. Nearly anhydrous formic-H² acid-H² (Note 1) is prepared by the thermal decomposition¹ of anhydrous oxalic-H₂² *in vacuo* at 180°. The product is purified by several vacuum distillations after pumping off the carbon dioxide (Note 2).

(c) *Formic-H² Acid*. The carboxyl deuterium atom is removed by treating formic-H² acid-H² several times with ordinary water. Anhydrous copper sulfate is used to dehydrate the acid between exchanges.

B. Notes

1. Preliminary tests with anhydrous oxalic acid indicated that formic acid with less than 3% water could be obtained.
2. The infrared spectrum of the product indicated that the CH² and OH² groups were at least 95% pure.

C. Other Preparations

Oxalic acid-H₂² has been prepared by the reaction² of barium oxalate with a solution of sulfur trioxide in water-H₂² and on several occasions by exchange with water-H₂² (see Table XVI, 12).

Formic- H^2 acid- H^2 has been prepared³⁻⁵ by the pyrolysis of oxalic acid- H^2 . Formic- H^2 acid has been obtained by exchange of formic- H^2 acid- H^2 with ordinary water.³

Formic- H^2 acid has also been prepared,⁶ as a 70% aqueous solution, by the hydrolysis of potassium cyanide (1.96 moles) with an excess of water- H^2 (10 moles) containing sodium hydroxide (0.1 mole) in a bomb tube at 175° .⁷

¹D. E. Wobbe and W. A. Noyes, Jr., *J. Am. Chem. Soc.*, **48**, 2856 (1926).

²I. Lütgert and E. Schröer, *Z. physik. Chem.*, **A187**, 133 (1940); through *Chem. Abstracts*, **35**, 4269 (1941).

³D. W. G. Style and J. C. Ward, *J. Chem. Soc.*, 1952, 2125.

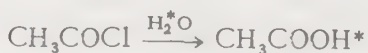
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⁵V. du Vigneaud, J. P. Chandler, S. Simmonds, A. W. Moyer and M. Cohn, *J. Biol. Chem.*, **164**, 603 (1946).

⁶D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, *J. Biol. Chem.*, **213**, 281 (1955).

⁷H. Sulzer, *Angew. Chem.*, **25**, 1268 (1912).

ACETIC ACID- H^2



H. Linschitz, M. E. Hobbs and P. M. Gross, *J. Am. Chem. Soc.*, **63**, 3234 (1941).

A. Procedure

Acetic acid- H^2 is prepared according to the procedure of Engler.¹ To 85 ml. of refractionated reagent-grade acetyl chloride (b.p. $50.08-50.13^\circ$ at 750 mm.) in a Claisen flask is added 18 ml. of water- H^2 (99.6%) through a dropping funnel. The apparatus is protected from atmospheric moisture by phosphorus pentoxide tubes, and dry nitrogen is bubbled through the mixture, during the reaction and the distillation, to sweep out hydrogen- H^2 chloride and excess acetyl chloride. After two successive reduced-pressure distillations, the acetic acid- H^2 is repeatedly fractionated through a 3-foot Widmer column and finally subjected to two fractional crystallizations (Note 1). Assuming all the impurity present to be water, the product is refluxed with the calculated amount of acetic anhydride and an oxidation catalyst, chromium trioxide, according to the method of Orton and Bradfield² (Note 2). The main fraction of the product, 17 ml., is sealed in an ampoule (Note 3).

B. Notes

1. A product free of chloride ions resulted which froze at $14.93-15.05^\circ$. Titration indicated 99.35% acetic acid, calculated as CH_3COOH^2 .

2. A sample of 98.54% acetic acid- H^2 is raised to 99.8%, m.p. 16.35° , in this way.

3. The acetic acid- H^2 thus obtained has the following physical properties: m.p. $15.66 \pm 0.05^\circ$, d_4^{30} 1.0527, d_4^{25} 1.0588, n_D^{20} 1.37102.

C. Other Preparations

Acetic acid- H^2 has been prepared by the reaction³ of anhydrous potassium acetate with concentrated sulfuric acid- H_2^2 ; from silver acetate and dry hydrogen- H^2 chloride;⁴ by the reaction of acetic anhydride^{5-7,13} with water- H_2^2 ; and by the reaction of acetyl chloride^{8-10,14} and water- H_2^2 .

Trichloroacetic acid- H^2 , propionic acid- H^2 and valeric acid- H^2 have also been prepared⁸ by the latter method, from the corresponding acyl chlorides.

Trifluoroacetic acid- H^2 has been prepared^{11,12} by the hydrolysis of bis(trifluoroacetic) anhydride with water- H_2^2 .

¹W. Engler, Z. physik, Chem., B32, 471 (1936).

²K. J. P. Orton and A. E. Bradfield, J. Chem. Soc., 1927, 983.

³J. O. Halford and L. C. Anderson, J. Am. Chem. Soc., 58, 736 (1936).

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⁶J. D. Roberts, C. M. Regan and I. Allen, J. Am. Chem. Soc., 74, 3679 (1952).

⁷G. E. Hall, R. Piccolini and J. D. Roberts, *ibid.*, 77, 4540 (1955).

⁸D. Hadzi and N. Sheppard, Proc. Roy. Soc. (London), A216, 247 (1953).

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¹¹N. Fuson, M. Josien, E. A. Jones and J. R. Lawson, J. Chem. Phys., 20, 1627 (1952).

¹²W. Klemperer and G. C. Pimentel, *ibid.*, 22, 1399 (1954).

¹³W. Weltner, Jr., J. Am. Chem. Soc., 77, 3941 (1955).

¹⁴A. E. Potter, Jr. and H. L. Ritter, J. Phys. Chem., 58, 1040 (1954).

ACETIC- H_3^2 ACID



J. O. Halford and L. C. Anderson, J. Am. Chem. Soc., 58, 736 (1936).

A. Procedure

A cold solution of acetic- H_3^2 acid- H^2 (Note 1) is treated with dry hydrogen chloride (Note 2). Progress of the reaction is checked from time to time by means of the melting point, after removal of hydrogen chloride under vacuum. When there is no further increase in the melting point of the product (17.2°) the exchange is considered to be complete (Note 3).

B. Notes

1. Acetic- H_3^2 acid- H^2 is prepared either from malonic- H_2^2 acid- H_2^2 or by exchange with water- H_2^2 .

2. The temperature is kept low to increase solubility and to minimize evaporation loss, which is checked by means of a cold trap.

3. Halford and Anderson compare the effects of varying degrees of deuterium substitution on the melting points of several aliphatic acids, for example: acetic- H_3^2 acid melts at 17.2° , acetic- H_3^2 acid- H^2 at $15.8-16^\circ$, and acetic acid at 16.7° . A theoretical discussion of the effects involved is given by these authors.

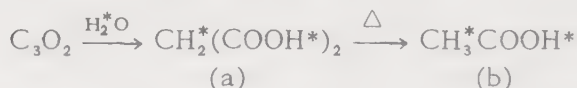
C. Other Preparations

Acetic- H_3^2 acid has been prepared by the reaction of barium acetate- H_2^2 and concentrated sulfuric acid¹ and by the simultaneous exchange and hydrolysis of acetonitrile in alkaline water- H_2^2 .^{2,3} According to Reitz³ the rate of exchange is 30-40 times the rate of hydrolysis of the nitrile group.

¹A. Dadieu and W. Engler, *Naturwissenschaften*, 24, 318 (1936).

²L. D. C. Bok and K. H. Geib, *Z. physik. Chem.*, A183, 353 (1939); through Chem. Abstracts, 33, 3240 (1939).

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ACETIC- H_3^2 ACID- H^2 

C. L. Wilson, *J. Chem. Soc.*, 1935, 492.

A. Procedure

(a) *Malonic- H_2^2 Acid- H_2^2* . The carbon suboxide¹ (6.3 ml. at 0° ; d_4^0 1.114), from 50 g. of diacetyltartaric anhydride,² is distilled into a graduated tube cooled to -78° (Note 1). About 1 g. of water- H_2^2 is weighed into a Pyrex tube (1.5×25 cm.), which is sealed at one end, and frozen at -78° . An equivalent amount of carbon suboxide, measured by volume, is then distilled into the tube, dry benzene (10 ml. per g. of water- H_2^2) is added, and the tube is sealed and shaken mechanically at room temperature (Note 2). After several days, the tube is opened, and the crystals of malonic acid are collected, washed with dry benzene and dried over phosphorus pentoxide at 0.01 mm. Malonic- H_2^2 acid- H_2^2 , m.p. $128-130^\circ$ (dec.), is obtained in nearly quantitative yield (Note 3).

(b) *Acetic- H_3^2 Acid- H^2* . Using a vacuum manifold equipped with a series of three 10-ml. bulbs, the malonic- H_2^2 acid- H_2^2 is thoroughly dried at 100°

and 0.01 mm., in the first bulb. When the temperature of the bath is raised to 140–150°, decomposition begins and acetic-H₃ acid-H² is collected in the second bulb cooled to 0°. When the decomposition is complete, the first bulb is sealed off, the the product is purified by vacuum distillation at 30° into the third bulb cooled to 0°. The product melts at 15.75–15.76° (Note 4).

B. Notes

1. Dissolved carbon dioxide is removed from the carbon suboxide by warming it to 0° for a few minutes. The suboxide is stable at –78° over periods of several days, and in benzene solution it can be kept at the ordinary temperature for several weeks without polymerization.

2. The benzene was dried over phosphorus pentoxide, then with carbon suboxide and distilled.

3. The melting points of malonic acid from 20% and 99.5% water-H₂ were, respectively, 131–132.5° and 128–130°.

4. These are the melting points of two separate preparations. Ordinary acetic acid prepared in the same manner melted at 16.65°.

C. Other Preparations

Malonic-H₂ acid-H₂ has been prepared from carbon suboxide and water-H₂, according to the method described,³⁻⁵ without a solvent,⁶ with carbon tetrachloride as solvent,⁷ and by exchange with water-H₂.^{6,8,12,13}

Acetic-H₃ acid-H² has been prepared by the thermal decomposition of malonic-H₂ acid-H₂ on a number of occasions.^{3-7,10-13} Potter and Ritter⁷ obtained a 53% yield, based on carbon suboxide, after the product was twice distilled through a Piros-Glover microstill (about 25 theoretical plates) and then fractionally crystallized 3 times; m.p. 15.92 ± 0.07° (95 ± 3 atom per cent deuterium). They also measured the vapor pressures of acetic acid and acetic-H₃ acid-H² at temperatures ranging from 24.34° to 124.39° and the density of acetic-H₃ acid-H² at temperatures from 28.70° to 113.32°.

Sodium acetate-H₃ has been prepared¹³ by exchanging the hydrogen atoms of sodium acetate with water-H₂ at 150° in a sealed tube.

¹C. D. Hurd and F. D. Pilgrim, *J. Am. Chem. Soc.*, **55**, 757 (1933).

²A. Wohl and C. Osterlin, *Ber.*, **34**, 1139 (1901).

³W. R. Angus, A. H. Leckie and C. L. Wilson, *Proc. Roy. Soc. (London)* **A155**, 183 (1936).

⁴J. D. Roberts and C. M. Regan, *J. Am. Chem. Soc.*, **74**, 3695 (1952).

⁵M. Corval and C. Piolet, *Bull. soc. chim. France*, **21**, 234 (1954).

⁶J. O. Halford and L. C. Anderson, *J. Am. Chem. Soc.*, **58**, 736 (1936).

⁷A. E. Potter, Jr., and H. L. Ritter, *J. Phys. Chem.*, **58**, 1040 (1954).

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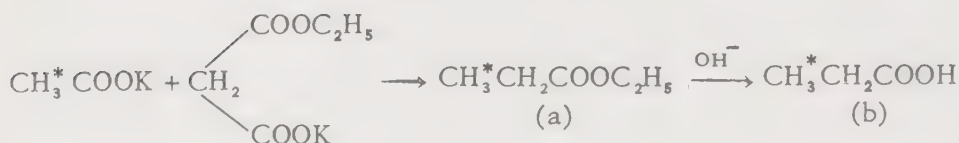
¹⁰R. C. Herman and R. Hofstadter, *ibid.*, 7, 460 (1939).

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¹²K. Clusius and H. Knopf, *Z. Naturforsch.*, 2b, 169-173 (1947).

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PROPIONIC-3-H₃ ACID



P. Hölemann and K. Clusius, *Ber.*, 70, 819 (1937).

A. Procedure

(a) *Ethyl Propionate-3-H₃* (Note 1). The electrolysis is carried out in a 10-ml. capacity U-tube such that frothing over is avoided. The anode and cathode spaces are separated by means of a glass-wool plug. The two platinum electrodes are 1 mm. thick and 15 mm. long. The anode electrolyte is composed of 1.7 g. of potassium acetate-H₃ and 1.7 g. of potassium ethyl malonate in 3 g. of water (Note 2). The cathode electrolyte is 4.5 ml. of a 25% solution of potassium carbonate. To suppress the possible wandering of hydroxyl ions into the anode space, a slow stream of carbon dioxide is introduced near the cathode through a fine capillary during the electrolysis. The synthesis is carried out with ice-cooling and a current strength of 0.25 ampere and is complete in about 3 hours. The oily droplets of crude ester, which weigh 0.65 g. (37% based on potassium acetate-H₃), float on the anode electrolyte solution (Note 3). The crude ester is separated from traces of salts by vacuum distillation and is then fractionated under vacuum by distillation from a bath at 0° and with careful observation of the vapor pressures (Note 4). The material with vapor pressure above 8 mm. is removed as a forerun. The middle fraction, which amounts to about 40% of the crude ester and has a vapor pressure of not less than 7 mm., is collected for hydrolysis.

(b) *Propionic-3-H₃ Acid*. The distillate of ethyl propionate-3-H₃ is hydrolyzed with 10% potassium hydroxide solution, and the excess alkali is neutralized with carbon dioxide. The alcohol from hydrolysis of the ester is removed under vacuum, and the residue is treated with an excess of pure oxalic acid in 3 ml. of water (Note 5). The propionic-3-H₃ acid is distilled from the residue of potassium oxalate under vacuum, and the succinic acid remains in the residue. The aqueous acid solution so obtained is distilled several times under vacuum to remove traces of oxalic acid.

B. Notes

1. The synthesis of propionic-3- H_3^2 acid is based upon the observation of Miller and Hofer¹ that ethyl propionate is formed at the anode by the electrolysis of a solution containing potassium acetate and potassium ethyl malonate.

2. The total volume is about 4.5 ml.

3. The crude ester contains a considerable amount of ethyl succinate and some ethyl acetate. In addition there are small amounts of methyl propionate, succinate and acetate formed as by-products by an ester interchange at the anode.

4. The separation of the mixture of esters is fairly good, since at 0° the vapor pressures of the components are as follows: methyl acetate, 62 mm.; ethyl acetate, 24 mm.; methyl propionate, 21 mm.; ethyl propionate, 8 mm.; ethyl succinate, about 3 mm.

5. Sulfuric acid was not used because of the possibility of hydrogen exchange.

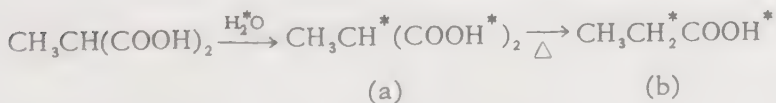
C. Other Preparations

Propionic-3- H_3^2 acid has been prepared² by the decarboxylation (see propionic-2- H_2^2 acid- H^2) of methyl- H_3^2 -malonic acid. The latter compound was obtained by hydrolysis of ethyl methyl- H_3^2 -malonate prepared by alkylation of ethyl malonate with methyl- H_3^2 bromide, essentially according to the procedure of Weiner.³

¹W. V. Miller and H. Hofer, Ber., 28, 2427 (1895).

²B. Nolin, Can. J. Chem., 31, 1257 (1953).

³Organic Syntheses, Coll. Vol. II, Wiley, New York, 1948, p. 279.

PROPIONIC-2- H_2^2 ACID- H^2 

P. Hölemann and K. Clusius, Ber., 70, 819 (1937).

A. Procedure

(a) *Methylmalonic-2- H^2 Acid- H_2^2* . In a sealed tube, 2.5378 g. of methylmalonic acid in 2.0642 g. of water- H_2^2 (99.21%) is heated for 8 hours at 55°. When the solution is cooled to 0°, most of the acid crystallizes, and the water is distilled off under vacuum (Note 1). The residue is again dissolved in water- H_2^2 , heated at 55°, and then is dried under vacuum (Note 2).

(b) *Propionic-2-H₂² Acid-H²*. The methylmalonic-2-H² acid-H₂² is decarboxylated by heating it to 135° under vacuum. The product is then vacuum-distilled (Note 3).

B. Notes

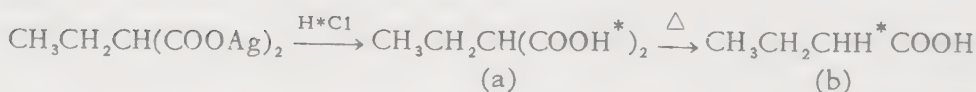
1. The water-H₂², which was collected over 20 mg. of fused sodium acetate to remove traces of acid and redistilled, had a density corresponding to 75.72% H₂O. Assuming the partition coefficient between water-H₂² and the organic compound to be one, the calculated number of exchangeable hydrogen atoms is 3.02. The hydrogen atom of the methine group is easily exchanged also.

2. After the second exchange, the deuterium content of the exchangeable hydrogen atoms was 95%.

3. Kruis and Schanzer¹ prepared 27 g. of propionic-2-H₂² acid-H² with a deuterium content of 99.45% in the 2-position, using the method of Hölemann and Clusius but employing a larger number of exchanges.

¹A. Kruis and W. Schanzer, *Z. physik. Chem.*, 191A, 301 (1942).

BUTYRIC-2-H₁² ACID



D. J. G. Ives and M. R. Nettleton, *J. Chem. Soc.*, 1948, 1085.

A. Procedure

(a) *Ethylmalonic Acid-H₂²*. Silver ethylmalonate is suspended in dry ether with vigorous stirring and treated with an equivalent amount of dry hydrogen-H² chloride in ether. When the reaction is complete in about one hour, the ether solution is transferred with a filter stick into a large sintered glass filter. The apparatus is enclosed and protected from moisture. The filtrate is evaporated under reduced pressure, leaving a product which melts at 107–109°.

(b) *Butyric-2-H₁² Acid*. The acid brucine salt of ethylmalonic acid-H₂² is prepared by adding 0.5 molar equivalent of brucine, dissolved in ethylene chloride, to a stirred solution of the acid-H₂² in ether. After one hour, the solvent is removed from the crystalline salt at room temperature, under partial vacuum. Decarboxylation is effected, *in vacuo*, below the melting point of the salt during 20 hours, with a maximum temperature of 150°. The butyric-2-H₁² acid-H², which distills, is col-

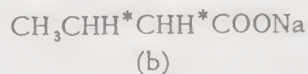
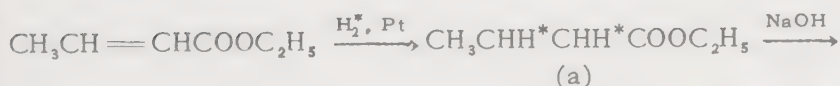
lected in a trap cooled with Dry Ice. The distilled sample, dissolved in alkali, is purified by a 12-hour continuous extraction with chloroform. The alkaline solution is then acidified with mineral acid. The product is continuously extracted with ether and, after removal of ether, is fractionally distilled; b.p. 85.5° (40 mm.). A small optical activity in the product is reduced by distillation at 0° under vacuum.

B. Other Preparations

A mixture of methyl butyrate-2- H_2^2 (67.0%), methyl butyrate-2- H_1^2 (24.0%) and methyl butyrate has been prepared¹ by the esterification of butyric-2- H_1^2 acid obtained from the decarboxylation of ethylmalonic-2- H^2 acid- H_2^2 . The latter compound was obtained by an exchange reaction with water- H_2^2 during 1 month.

¹K. B. Wiberg, J. Am. Chem. Soc., 74, 4382 (1952).

SODIUM BUTYRATE-2,3- H_2^2



D. Rittenberg, R. Schoenheimer and E. A. Evans, J. Biol. Chem., 120, 503 (1937).

A. Procedure

(a) *Ethyl Butyrate-2,3- H_2^2* . Ethyl crotonate, 12.1 g., is hydrogenated with hydrogen- H_2^2 in the presence of platinum oxide catalyst¹ without the addition of a solvent. After one mole of hydrogen- H_2^2 per mole of ethyl crotonate is absorbed, the product is isolated by distillation *in vacuo*.

(b) *Sodium Butyrate-2,3- H_2^2* . The ethyl butyrate-2,3- H_2^2 is hydrolyzed by heating under reflux with 7% alcoholic sodium hydroxide solution. Then, 50 ml. of water is added, and carbon dioxide is bubbled through until the solution is neutral. After the solution is concentrated to dryness, the residue is extracted with alcohol. The alcohol-soluble fraction, sodium butyrate-2,3- H_2^2 , is twice recrystallized from ethanol.

(c) *Sodium Hexanoate-2,3,4,5- H_4^2* . The sodium salt of hexanoic-2,3,4,5- H_4^2 acid is prepared by the hydrogenation of ethyl sorbate (ethyl 2,4-hexadienoate), followed by alkaline hydrolysis as in the preparation of sodium butyrate-2,3- H_2^2 .

B. Other Preparations

Sodium butyrate-2,3- H_2^2 has been prepared by Morehouse² in the manner described. Sodium butyrate-3,4- H_2^2 was also prepared by Morehouse by the hydrogenation of ethyl 3-butenate. The latter compound was prepared by the method of Bruylants³ as modified by Linstead.⁴

Butyric acid- H^2 has been prepared⁵ by the hydrolysis of butyryl chloride with water- H_2^2

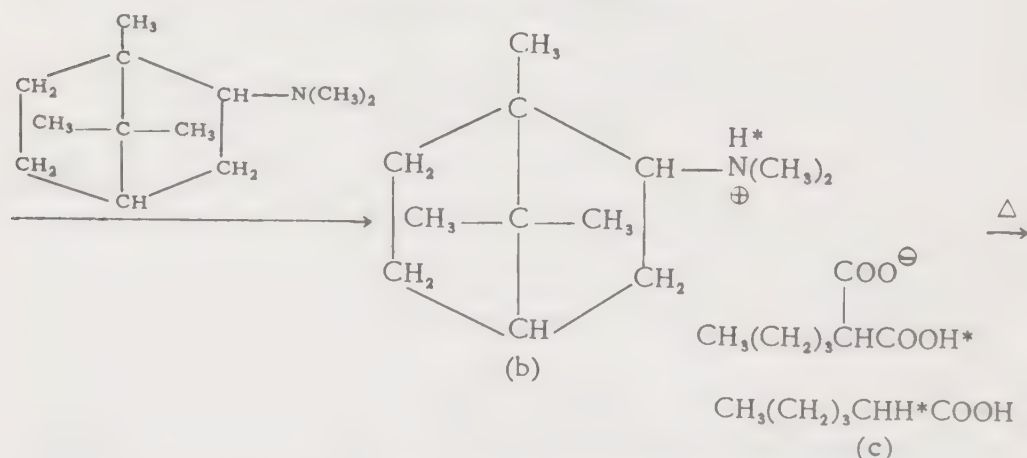
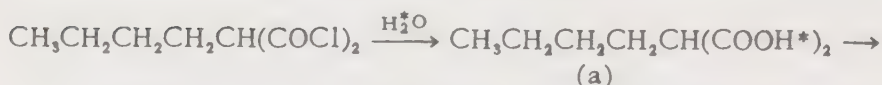
¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

²M. G. Morehouse, *J. Biol. Chem.*, 129, 769 (1939).

³P. Bruylants, *Bull. soc. chim. Belges*, 38, 133 (1929).

⁴R. P. Linstead, E. D. Noble and E. J. Boorman, *J. Chem. Soc.*, 1933, 557.

⁵R. C. Herman, *J. Chem. Phys.*, 8, 252 (1940).

HEXANOIC-2- H_1^2 ACID

D. J. G. Ives and M. R. Nettleton, *J. Chem. Soc.*, 1948, 1085.

A. Procedure

(a) *Butylmalonic Acid- H_2^2* . Butylmalonyl chloride dissolved in ether is treated with an equivalent amount of water- H_2^2 (Note 1).

(b) *N,N-Dimethylbornylammonium-N- H^2 Hydrogen- H^2 Butylmalonate*. An ether solution of (+)-*N,N*-dimethylbornylamine (Note 2), in slight deficiency of one-half equivalent based on the acid, is added slowly to a stirred solution of butylmalonic acid- H_2^2 in ether. Ether is removed from the precipitated crystalline acid salt at room temperature.

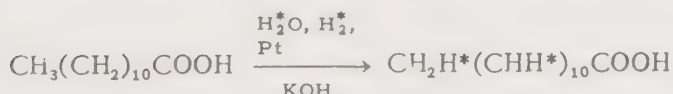
(c) *Hexanoic-2- H_1^2 Acid*. The above acid salt is decarboxylated, under vacuum, by heating it at a maximum temperature of 125° . The residue is dissolved in alkali and extracted several times with ether. The solution

is then acidified with mineral acid; the product is extracted with ether and dried. After removal of ether, the hexanoic-2- H_1^2 acid is distilled under reduced pressure, b.p. 98° (9 mm.) (Note 3).

B. Notes

1. A special procedure for the preparation of butylmalonyl chloride is described by Ives and Nettleton.
2. The preparation of (+)-*N,N*-dimethylbornylamine and of (+)-bornylamine hydrochloride is described by Ives and Nettleton.
3. The final product was optically inactive.

H^2 -LAURIC ACID



W. E. van Heyningen, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **125**, 495 (1938).

A. Procedure (Note 1)

In a long-necked flask, 750 mg. of platinum oxide catalyst¹ suspended in 7.5 ml. of water- H_2^2 (99.5%) is reduced with hydrogen- H_2^2 . To the suspension of reduced catalyst are added 7.5 g. of lauric acid and 100 mg. of potassium hydroxide. After the flask is cooled with Dry Ice and evacuated and sealed, it is shaken for 6 days at 130 – 135° . The water- H_2^2 is removed *in vacuo*, the residue is acidified by addition of phosphorus pentoxide, and the H^2 -lauric acid is extracted into ether. The product is extracted from the ethereal solution with dilute alkali and again extracted into ether after acidification of the base (Note 2). On recrystallization of the product from a large volume of aqueous acetone, 7.2 g. of H^2 -lauric acid, m.p. 45.2° , is obtained (Note 3).

Other fatty acids prepared by the above procedure are listed in Table XVI, 1.

B. Notes

1. This is a general procedure for preparing saturated fatty acids containing deuterium. Apparently a random distribution of deuterium results.
2. This is done to replace the labile deuterium of the hydroxyl group with hydrogen.
3. The product contained 28.7 atom per cent deuterium. If complete equilibrium had been attained (excluding the carboxyl hydrogen) the deuterium content of the isolated fatty acid would have been 48.1 atom

TABLE XVI, 1

H²-Fatty Acids Prepared by Hydrogen Exchange

Acid	Grams	Deuterium content, atom %	Calculated atom % deuterium at equilibrium	Exchange %
a. Stearic	7.5	22.4 ± 0.2	45.5	49.2
Palmitic	5.0	21.9 ± 0.4	55.5	39.5
Lauric	7.5	28.7 ± 0.4	46.2	62.1
b. Myristic	7.5	29.8 ± 0.3	34.0	87.5
Decanoic	7.5	13.9 ± 0.3	34.3	40.6
Octanoic	7.5	25.1 ± 0.4	34.4	73.0
c. Myristic	7.5	4.40 ± 0.1	29.0	15.2
Decanoic	7.5	1.49 ± 0.1	29.5	5.1
Octanoic	7.5	5.20 ± 0.1	29.6	17.6

a. 750 mg. PtO₂ · H₂O, 100 mg. KOH in 7.5 ml. of 99.5% water-H₂ at 130° for 6 days.

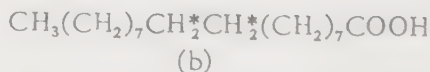
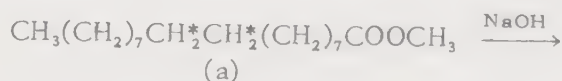
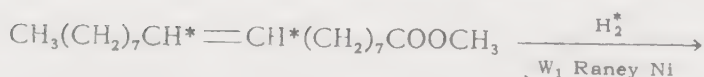
b. 1.5 g. PtO₂ · H₂O, 100 mg. KOH in 15 ml. of 55% water-H₂ at 130° for 12 days.

c. 100 mg. PtO₂ · H₂O, 100 mg. KOH in 10 ml. of 55% water-H₂ at 130° for 6 days.

per cent. The deuterium thus introduced is not removed by treatment of the fatty acids with dilute mineral acid or dilute alkali at about the boiling point of ethanol.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

OCTADECANOIC-9,10-H₂ ACID
(Stearic-9,10-H₂ Acid)



N. A. Khan, F. E. Deatherage and J. B. Brown, *J. Am. Oil Chemists Soc.*, 28, 27 (1951).

A. Procedure

(a) *Methyl Octadecanoate-9,10-H₂*. Methyl oleate-9,10-H₂ is treated with hydrogen-H₂ at room temperature and atmospheric pressure over a

Raney nickel W_1 catalyst (see methyl oleate-9,10- H_2^2). The yield of saturated ester, m.p. $38-38.5^\circ$, is 98-99%.

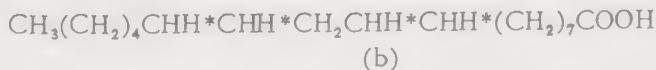
(b) *Octadecanoic-9,10- H_4^2 Acid*. Methyl octadecanoate-9,10- H_4^2 is saponified with dilute sodium hydroxide heated under reflux. Acidification of the cooled solution of the sodium salt precipitates the free acid, which is collected and washed with cold water. After recrystallization the octadecanoic-9,10- H_4^2 acid melts at $69-69.2^\circ$.

B. Other Preparations

Schoenheimer and Rittenberg¹ prepared methyl stearate and stearic acid, containing deuterium, by shaking the methyl esters of fatty acids from linseed oil with platinum oxide catalyst and hydrogen- H_2^2 until complete saturation occurred. The crystalline product was saponified with potassium hydroxide in methanol, and the acid was recrystallized from dilute ethanol.

¹R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, **113**, 505 (1936).

OCTADECANOIC-9,10,12,13- H_4^2 ACID



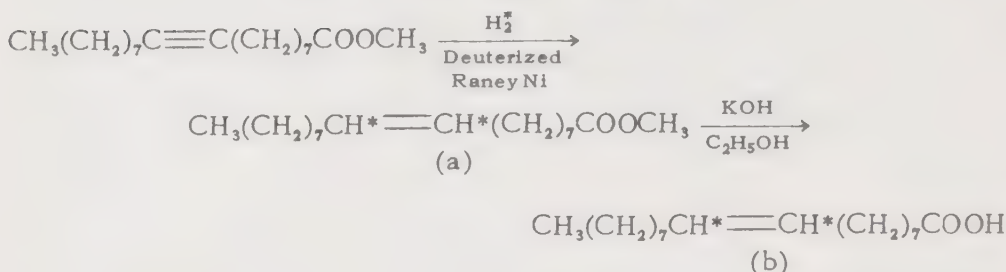
R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, **111**, 167 (1935).

A. Procedure

(a) *Methyl Octadecanoate-9,10,12,13- H_4^2* . Methyl linoleate, 4.5 g., dissolved in dry petroleum ether, is shaken at room temperature in an atmosphere of hydrogen- H_2^2 with 100 mg. of platinum oxide catalyst. After 85 minutes the absorption of hydrogen- H_2^2 ceases. The catalyst is collected and washed with petroleum ether. The combined filtrate is evaporated to dryness.

(b) *Octadecanoic-9,10,12,13- H_4^2 Acid*. The crude methyl ester is heated under reflux with dilute sodium hydroxide until dissolution is complete. The solution is acidified with mineral acid, cooled and extracted with ether. After removal of ether, the crude acid is recrystallized from alcohol, m.p. 69° .

OLEIC-9,10- H_2^2 ACID
 (*cis*-9-Octadecenoic-9,10- H_2^2 Acid)



N. A. Khan, J. Am. Chem. Soc., 74, 3018 (1952).

A. Procedure (Note 1)

(a) *Methyl Oleate-9,10- H_2^2* , (*Methyl cis-9-Octadecenoate-9,10- H_2^2*). Methyl stearolate, 4.25 g., dissolved in purified dioxane, is hydrogenated with hydrogen- H_2^2 in the presence of 0.4 g. of deuterized Raney nickel (Note 2), at room temperature and atmospheric pressure, until 1.06 moles of hydrogen- H_2^2 per mole of substrate is absorbed. After removal of the catalyst with a fine sintered-glass funnel, the solvent is removed, under vacuum, from the crude product. The crude mixture is freed of saturated ester by crystallization at -32° from 5% acetone solution, then cooling the filtrate slowly to -45° and warming it to -36° , and finally cooling the filtrate to -50° and warming it to -36 to -38° . Methyl oleate-9,10- H_2^2 is then crystallized by cooling the final filtrate to -65° . The product is twice washed with the solvent at -65° and then distilled under vacuum, b.p. $173-174^\circ$ (1.8-2.0 mm.); iodine number, 84.9 (theory 85.23); n_D^{20} 1.45185 (Note 3).

(b) *Oleic-9,10- H_2^2 Acid*, (*cis-9-Octadecenoic-9,10- H_2^2 Acid*). A mixture of alcohol and potassium hydroxide, which has refluxed for 3 hours, is used to saponify 15 g. of methyl oleate-9,10- H_2^2 . After addition of distilled water, the solution is acidified with 6 N hydrochloric acid. The product is extracted with two portions of ether, which are combined, washed free of mineral acid, and dried; then the solvent is removed. The oleic-9,10- H_2^2 acid is distilled under vacuum, b.p. $185-186^\circ$ (1.8-2 mm.); neut. equiv. 283.5; deuterium, 5.64 atom per cent (theory 5.88) (Note 4).

B. Notes

1. In this extension of work previously reported,¹ a special deuterized, hydrogen-free Raney nickel catalyst was prepared which was also free of alkali and alumina, insofar as possible. The "ordinary" Raney nickel thus prepared and its deuterized form both proved to be more selective

for the hydrogenation of stearolic acid and its methyl ester to the corresponding olefinic compounds than W_1 ,² and different from W_1 , W_2 and W_3 .³

2. In preparing the deuterized Raney nickel catalyst, Raney nickel was digested according to the procedure of Adkins,⁴ except that the temperature was kept between 100–105°. The catalyst was washed in an open cylinder provided with a stirrer and a water inlet tube reaching to the bottom of the cylinder. By adjustment of water flow and stirring speed, the catalyst was suspended and washed until the water was neutral. Additional light particles were removed by agitating the catalyst in 1 l. of water at a time and then decanting the turbid water layer. This process was repeated until no turbidity appeared. The alkali-free catalyst was washed with 250 ml. of purified dioxane and then covered with 1.5 l. of dioxane, which was distilled until the temperature of the vapor reached 101°. This "ordinary" Raney nickel proved to be highly selective for the hydrogenation of a triple bond.

The "ordinary" Raney nickel, 25 g., (still wet with dioxane) was washed with 25 ml. of dioxane by centrifugation and then suspended in 10 ml. of water- H_2 (99.9%) in a stoppered tube for 48 hours. The catalyst was stirred occasionally throughout the equilibration period. It was then washed with three 25-ml. portions of dioxane and transferred to the reaction vessel of a Joshel apparatus⁵ (250-ml.) with the help of 125 ml. of dioxane. Five ml. of water- H_2 was introduced into the reaction vessel; the stopcock above the vessel was closed, the system was evacuated, and hydrogen- H_2 gas was introduced. The catalyst in dioxane was agitated under a slight pressure of hydrogen- H_2 for 2 hours. The process was repeated 3 times, the system being flushed each time with dry, oxygen-free nitrogen and filled with fresh hydrogen- H_2 . The catalyst prepared in this manner was stored in purified dioxane containing a small amount of water- H_2 .

3. The deuterium content of the ester was 5.45 atom per cent (theory 5.55 atom per cent).

4. Calculations based on the fact that four atoms of hydrogen but only two atoms of iodine (Wijs 0.5-hour method) add to the acetylenic bond, while two atoms of either hydrogen or iodine add to ethylenic bonds, indicated that the crude product was composed of: methyl octadecanoate-9,10- H_4 , 2.9%; methyl stearolate, 2.5%; and methyl oleate-9,10- H_2 , 94.6%. Infrared absorption measurements at $10.36 \mu^6$ indicated the latter product to be a mixture of 95.8% *cis*- and 4.5% *trans*-isomers.

C. Other Preparations

Methyl oleate-9,10- H_2 , with 90% of the theoretical deuterium has been prepared,¹ in yields of 65–75%, using ordinary W_1 Raney nickel catalyst.

¹N. A. Khan, F. E. Deatherage and J. B. Brown, J. Am. Oil Chemists Soc., 28, 27 (1951).

²H. Adkins and L. W. Covert, J. Am. Chem. Soc., 54, 4116 (1932).

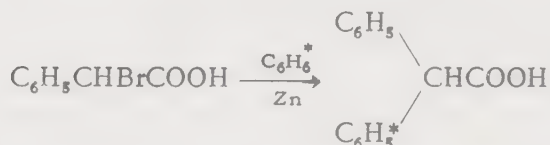
³A. A. Pavlic and H. Adkins, *ibid.*, 68, 1471 (1946).

⁴H. Adkins and L. W. Covert, *ibid.*, 54, 4116 (1932).

⁵L. M. Joshel, Ind. Eng. Chem., Anal. Ed., 15, 590 (1943).

⁶D. Swern, H. G. Knight, O. D. Shreve and M. R. Heether, J. Am. Oil Chemists Soc., 27, 17 (1950).

PHENYLPHENYL-H₅²-ACETIC ACID



H. Erlenmeyer and H. Schenkel, Helv. Chim. Acta, 19, 1169 (1936).

A. Procedure

Phenylphenyl-H₅²-acetic acid is prepared according to the procedure of Symons and Zincke,¹ from L-bromophenylacetic acid and benzene-H₆². One part of bromophenylacetic acid is dissolved in two parts of benzene; the solution is warmed on a water-bath, and zinc dust is added in small amounts as long as hydrogen is evolved (Note 1). Then the mixture is heated further for a short time and cooled. After removal of the unreacted benzene, the residue is heated with sodium carbonate solution and filtered. The filtrate is acidified with hydrochloric acid, and the organic acid, which first appears as an oil and solidifies, is collected and washed with water. In order to purify the product it is dissolved in barium hydroxide solution, which is then treated with carbon dioxide and filtered. The filtrate is concentrated until the barium salt of diphenylacetic acid crystallizes (Note 2). The barium salt is dissolved in alcohol and filtered to remove an insoluble barium salt (Note 3). Upon standing, the solution deposits large monoclinic crystals which contain alcohol of crystallization and soon disintegrate in air. From these crystals diphenylacetic acid of constant melting point, 145-146°, is obtained (Note 4). The compound crystallizes from water in fine needles and is easily soluble in alcohol, ether and chloroform.

B. Notes

1. The hydrogen results from the reaction of hydrogen bromide with zinc.

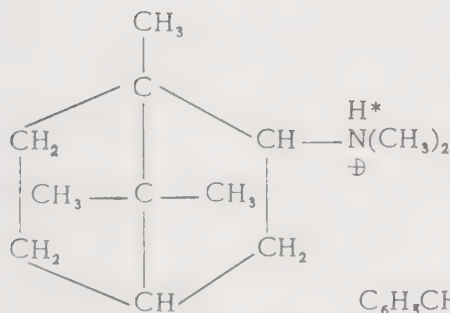
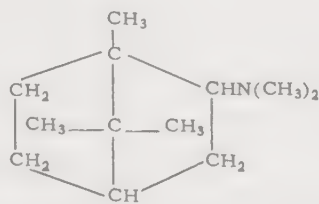
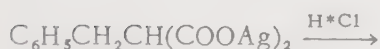
2. The product obtained upon treatment of the barium salt with dilute sulfuric acid was quite difficult to purify, and a constant-melting compound was not obtained by recrystallization of the product from water.

3. The compound, the barium salt of which was insoluble in alcohol, gave analyses corresponding to an α, α' -diphenylbenzenediacetic acid.

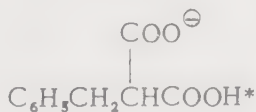
4. The product may also be purified by preparation of its ethyl ester directly from an alcoholic solution of the barium salt treated with dry hydrogen chloride.

¹R. Symons and Th. Zincke, *Ann.*, 171, 117 (1874).

HYDROCINNAMIC- α -H² ACID



(b)



(c)

D. J. G. Ives and M. R. Nettleton, *J. Chem. Soc.*, 1948, 1085.

A. Procedure

(a) *Benzylmalonic Acid-H₂²*. Silver benzylmalonate is suspended in dry ether with vigorous stirring, and a solution of hydrogen-H² chloride (1 molar equivalent) in dry ether is slowly added (Note 1). The resulting solution of benzylmalonic acid-H₂² is transferred through a coarse filter stick into a large enclosed sintered-glass filter. The filtrate is evaporated to dryness under reduced pressure, leaving a colorless crystalline residue of benzylmalonic acid-H₂², m.p., 119–122°; yield, 85–90%.

(b) *N,N-Dimethylbornylammonium-N-H² Hydrogen-H² Benzylmalonate*. An ether solution of *N,N*-dimethylbornylamine (Note 2), in slight deficiency of 0.5 equivalent based on the acid, is added slowly with stirring to an ether solution of benzylmalonic acid-H₂². Ether is removed from the precipitated salt at room temperature.

(c) *Hydrocinnamic- α -H₁² Acid, (3-Phenylpropionic-2-H₁² Acid)*. The above *N,N*-dimethylbornylammonium acid salt is decarboxylated in the course of 2.5 hours, by heating it under vacuum up to a maximum temperature of 110° (Note 3). The residue is dissolved in basic solution and repeatedly extracted with ether to remove impurities. The solution is then acidified with mineral acid, and the liberated product is extracted into ether. Hydrocinnamic- α -H₁² acid, m.p. 48–49°, is recovered from the ether (Note 4).

B. Notes

1. The preparation of the hydrogen-H² chloride solution starting with water-H₂² and phosphorus pentachloride is described by Ives and Nettleton.

2. The preparation of (+)-*N,N*-dimethylbornylamine is described by Ives and Nettleton.

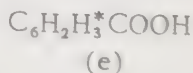
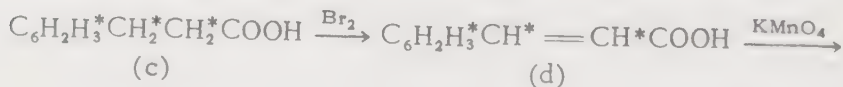
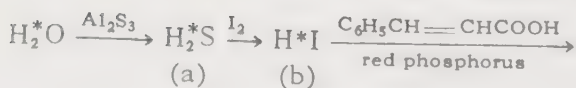
3. In this attempt to prepare an optically active compound, in which asymmetry is due to a deuterium atom, the Marckwald¹ synthesis of substituted acetic acids was employed. In this synthesis, the acid may be obtained under chemically mild conditions and in amounts sufficiently large to allow very careful purification. According to Eisenlohr and Meier² a well-defined crystalline salt is essential for a successful asymmetric synthesis; therefore efforts were made to achieve decarboxylation without melting the salt. In addition to the procedure already described, the salt was decarboxylated: at a maximum temperature of 80° during 8.5 hours; rapidly at 110° from a salt prepared without isolation of the acid; and during 13 days at a maximum temperature of 66°.

4. In addition to the *N,N*-dimethylbornylammonium salt of benzylmalonic acid-H₂², the brucine salt and the nicotine salt were also decarboxylated.

¹W. Marckwald, Ber., 37, 349 (1904).

²F. Eisenlohr and G. Meier, *ibid.*, 71, 997 (1938).

H²-BENZOIC ACID



H. Erlenmeyer and H. Gärtner, Helv. Chim. Acta, 19, 145 (1936); *ibid.*, 19, 331 (1936).

A. Procedure

(a) *Hydrogen-H₂² Sulfide*. In an enclosed apparatus, 5 g. of freshly prepared aluminum sulfide and 2 ml. of water-H₂² are warmed with stirring; toward the end of the reaction the temperature reaches 180°. The hydrogen-H₂² sulfide (about 1200 ml.) is collected in a gas buret over dry mineral oil.

(b) *Hydriodic Acid-H²*. In an all-glass apparatus and with shaking and outside ice-cooling, the hydrogen-H₂² sulfide is reacted with 6.78 g. of iodine suspended in 7.2 ml. of water-H₂². The hydriodic acid-H² is filtered to remove sulfur and is then freed of dissolved hydrogen-H₂² sulfide under vacuum.

(c) *H²-Hydrocinnamic Acid* (Note 1). According to the procedure of Gabriel and Zimmerman,¹ cinnamic acid, 3 g., is reduced with the above hydriodic acid-H² at 150° in the presence of 1 g. of red phosphorus. The reduction is carried out in an all-glass flask and condenser, with stirring, during 2.5 hours. The oily product, which solidifies upon cooling, is collected on a filter, dissolved in hot ammonium hydroxide, and filtered to remove phosphorus. The filtrate is cooled, and the crude H²-hydrocinnamic acid, precipitated by the addition of hydrochloric acid, is dissolved in dilute potassium carbonate solution. After a short period of steam distillation, the residual solution is cooled and acidified with hydrochloric acid. In this manner, 2.50 g. of H²-hydrocinnamic acid is obtained, which is further purified by dissolution in 200 ml. of petroleum ether (Note 2) and treatment with charcoal. The residue, remaining upon evaporation of the solvent, is recrystallized from petroleum ether to obtain 1.87 g. of H²-hydrocinnamic acid, m.p. 47° (Note 3).

(d) *H²-Cinnamic Acid*. The degradation is according to the procedure of Glaser.² In a glass apparatus, 0.11 ml. of bromine is introduced as vapor into 0.3 g. of H²-hydrocinnamic acid at 160°, during 30 minutes, with the aid of a slow stream of air. With the air still flowing, the reaction is completed during 45 minutes at 140°. The hydrogen bromide formed is collected in potassium carbonate solution. The resulting H²-cinnamic acid is dissolved in hot water, steam-distilled for a short time, treated with carbon and filtered. Upon cooling the filtrate, a small amount of crude acid is obtained (Note 4). The crude product is recrystallized from water to obtain 0.24 g. of H²-cinnamic acid, m.p. 133°. The pure nonisotopic acid and a mixture of the two melt at 133°.

(e) *H²-Benzoic Acid*. According to the procedure of Nicholls,³ 0.1 g. of H²-cinnamic acid is dissolved in 1.67 ml. of 1 N potassium hydroxide, 180 ml. of 0.1 N potassium permanganate solution is added, and the solution is kept tightly stoppered until the odor of benzaldehyde disappears (about 1 hour). Then, 40 ml. of 20% sulfuric acid is added, and after 20

minutes, destruction of formed oxalic acid is complete. With constant stirring, the acidic solution is added gradually to a solution of 9 ml. of 3% hydrogen peroxide in 20 ml. of water. In this manner the excess permanganate is destroyed, and the precipitate of manganese dioxide is brought into solution. The excess of hydrogen peroxide is titrated with 0.1 *N* potassium permanganate. The clear solution is extracted six times with ether (about 800 ml.), and the combined ether solution is washed four times with small quantities of water and dried over magnesium sulfate. Evaporation of the solvent leaves a white crystalline residue, which is dissolved in 1 ml. of 1 *N* sodium hydroxide solution and filtered through charcoal. The filtrate is acidified with hydrochloric acid and cooled to obtain crystals of H²-benzoic acid. The dry product is sublimed, *in vacuo*, at 130–150°; yield 0.05 g., m.p. 120.5°.

(f) *Strychnine H²-Hydrocinnamate*. Into a solution of H²-hydrocinnamic acid, obtained from 0.85 g. of H²-cinnamic acid, in 145 ml. of water is washed 1.89 g. of freshly precipitated strychnine. After it is warmed for a short time at 50°, the solution is filtered from a small amount of residue. Evaporation of a small part of this solution *in vacuo* to 0.5 volume furnished a mass of small seed crystals. The main solution is evaporated at 40° *in vacuo* for several days until no more of the seed crystals will dissolve (Note 5). During 6 days, clear, well-formed crystalline aggregates appear. This fraction of strychnine salt, 1.05 g., is collected, crushed, washed with water and dried over phosphrous pentoxide, m.p. 100° (not sharp). The mother liquor is concentrated *in vacuo* to 0.7 of its volume, and after a week a second fraction of 0.35 g. of the salt crystallizes, m.p. about 100°. After standing over calcium chloride for 4 weeks, the second mother liquor finally deposits 0.53 g. of the strychnine salt.

B. Notes

1. Erlenmeyer and Gärtner⁴ described a method of deuterium analysis which was subject to error of ±0.5%. Following degradation of the H²-hydrocinnamic acid successively to H²-cinnamic and H²-benzoic acid, they assigned the following formulae to these compounds, respectively: C₆H_{2.18}H_{2.82}C₂H_{1.62}H_{2.18}COOH, C₆H_{2.18}H_{2.82}C₂H_{0.74}H_{1.26}COOH and C₆H_{2.18}-H_{2.82}COOH.

2. B.p. 45–60°.

3. A mixture of the H²-hydrocinnamic acid with ordinary hydrocinnamic acid melted at 48°.

4. This entire process was repeated a second time, and the crude products were combined.

5. This point was reached when the volume of the solution was still 120 ml.

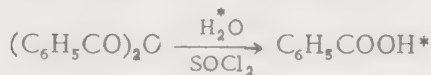
¹S. Gabriel and J. Zimmerman, *Ber.*, 13, 1680 (1880).

²A. Glaser, *Ann.*, 143, 345 (1867).

³J. R. Nicholls, *Analyst*, 53, 19, (1928).

⁴H. Erlenmeyer and H. Gärtner, *Helv. Chim. Acta*, 19, 129 (1936).

BENZOIC ACID-H²



J. M. Robertson and A. R. Ubbelohde, *Proc. Roy. Soc.*, (London) A170, 222, (1939).

A. Procedure

With precautions taken to exclude atmospheric moisture, 1 g. of benzoic anhydride is heated under reflux with 3 g. of water-H₂² (99.6%), with 1 drop of thionyl chloride added as catalyst. After the excess water is removed, the dry acid is sublimed in dry air (Note 1) and recrystallized by slowly cooling a solution of the acid in dry benzene (Note 2). The melting point of the benzoic acid-H² is 120.1° ± 0.5° (Note 3).

B. Notes

1. This quantity of benzoic acid is readily sublimed under vacuum.
2. Benzoic acid may be recrystallized from heptane with a very good return.
3. Benzoic acid prepared in the same manner melted at 123.4 ± 0.6°.

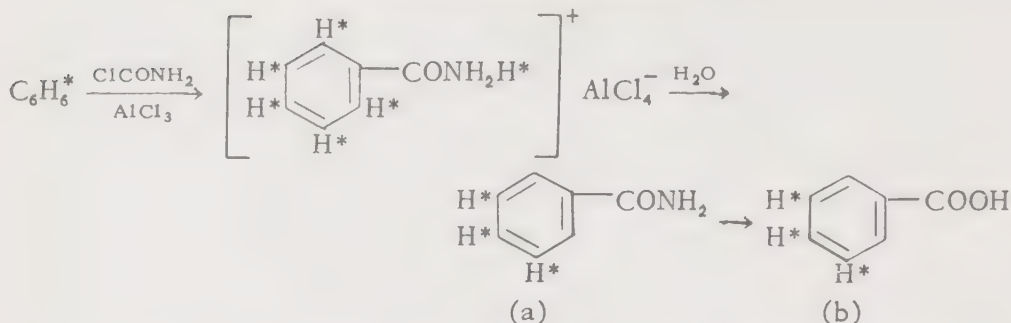
C. Other Preparations

Erlenmeyer¹ prepared benzoic acid-H² by exchange with warm water-H₂² (approximately 50%). The sublimed product melted at 118.5° (ordinary benzoic acid, m.p. 121.5°).

Benzoic acid-H² has also been prepared² by the reaction of water-H₂² with a slight excess of benzoyl chloride.

¹H. Erlenmeyer, A. Epprecht, H. Lobeck and H. Gärtner, *Helv. Chim. Acta*, 19, 354 (1936).

²D. Hadzi and N. Sheppard, *Proc. Roy. Soc. (London)*, A216, 247 (1953).

BENZOIC-3,4,5- H_3^2 ACID

H. Erlenmeyer, H. Lobeck, H. Gärtner and A. Epprecht, *Helv. Chim. Acta*, 19, 336 (1936).

A. Procedure

(a) *Benzamide-3,4,5- H_3^2* . According to the procedure of Gattermann,¹ benzamide is prepared by warming a mixture of carbamyl chloride, aluminum chloride and benzene in carbon disulfide. The aluminum chloride complex is decomposed with water, added cautiously, and then heated to boiling. After cooling the solution, the benzamide is collected, washed with water and dried (Note 1).

(b) *Benzoic-3,4,5- H_3^2 Acid*. The benzamide-3,4,5- H_3^2 is hydrolyzed to benzoic-3,4,5- H_3^2 acid by heating under reflux with potassium hydroxide solution. The cooled solution is acidified with concentrated hydrochloric acid. The precipitate of crystalline benzoic-3,4,5- H_3^2 acid is collected, dried, recrystallized from petroleum ether and sublimed under vacuum, m.p. 121.3°. The deuterium content is 2.94 atoms per mole (Note 2).

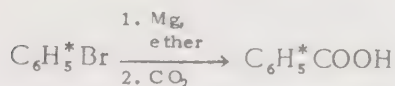
B. Notes

1. Benzene- H_6^2 was used in the above procedure. The benzamide was not analyzed for deuterium; the deuterium content and location in the molecule are based on the analysis of the benzoic-3,4,5- H_3^2 acid and the discussion given by Erlenmeyer, *et al.*

2. The benzoic-3,4,5- H_3^2 acid was analyzed by the method of Erlenmeyer and Gärtner.² Apparently two atoms of deuterium had exchanged with hydrogen of the water used in decomposing the aluminum chloride complex. Erlenmeyer, *et al.*, suggest that the equivalent *o*-positions in the aluminum chloride complex are activated and are the ones likely to exchange with water. Whatever the mechanism, it is suggested that loss of deuterium could be avoided by using water- H_2^2 in the hydrolysis of the aluminum chloride complex.

¹L. Gattermann, *Ann.*, 244, 50 (1888); *Ber.*, 32, 1117 (1899).

²H. Erlenmeyer and H. Gärtner, *Helv. Chim. Acta*, 19, 129 (1936).

BENZOIC- H_5^2 ACID

H. Erlenmeyer, H. Lobeck and A. Epprecht, *Helv. Chim. Acta*, 19, 793 (1936).

A. Procedure

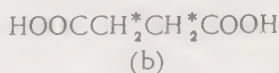
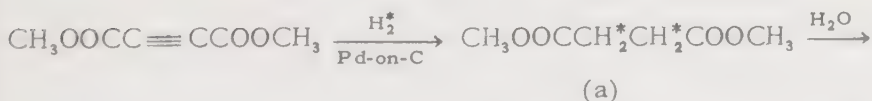
Phenylmagnesium- H_5^2 bromide is prepared from 0.35 g. of magnesium turnings and 2.3 g. of bromobenzene- H_5^2 in ether solution. With external cooling the Grignard reagent is carbonated and then hydrolyzed with dilute hydrochloric acid. The benzoic- H_5^2 acid is dissolved in ether, concentrated to dryness, recrystallized from water and finally sublimed under vacuum. The melting point of the benzoic- H_5^2 acid (Note 1) is 120.9° compared to normal benzoic acid, m.p. 121.7° (Note 2).

B. Notes

1. Isotopic analysis¹ indicated the composition of the acid to be $C_6H_{0.27}H_{4.73}^2COOH$.

2. Solubilities of the two compounds also differ slightly; at 18° a saturated solution of benzoic- H_5^2 acid in water contains 0.017 g./5 ml. The corresponding value for normal benzoic acid is 0.014 g./5 ml.

¹H. Erlenmeyer and H. Gärtner, *Helv. Chim. Acta*, 19, 129 (1936).

SUCCINIC- H_4^2 ACID

D. L. Williams and A. R. Ronzio, Atomic Energy Commission Report 2126; *Nuclear Sci. Abstr.*, 6, 5075 (1952).

A. Procedure

(a) *Methyl Succinate- H_4^2* . Into the small reaction flask, A (see Figure XVI, 1); is weighed 24.1 mg. of palladium catalyst (Note 1) and 145.3 mg. of methyl acetylenedicarboxylate. The flask is attached to the vacuum manifold and cooled with liquid nitrogen. The apparatus, including the gas buret and the pressure tubing connecting the cylinder of hydrogen- H_2^2 and the gas buret B, is then evacuated. The buret, manifold and reaction flask are filled with hydrogen- H_2^2 via the 3-way stopcock D; the initial

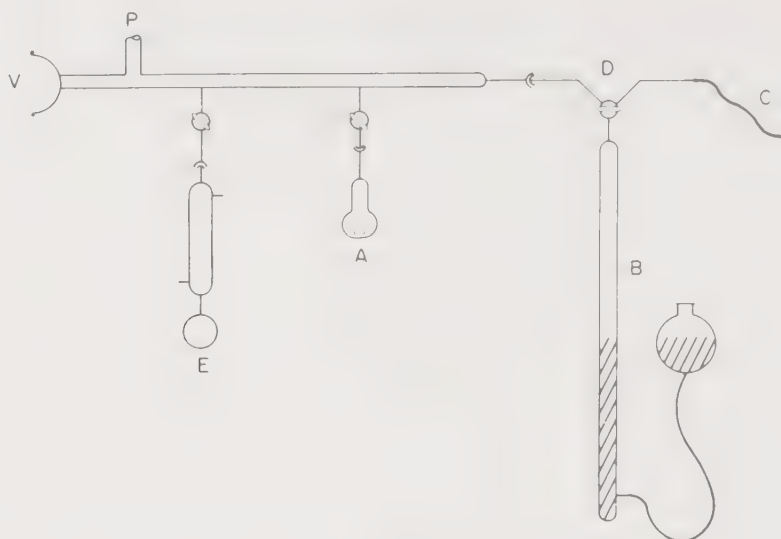


Fig. XVI, 1 Apparatus for the preparation of succinic- H_4^2 acid (D. L. Williams and A. R. Ronzio). A, reaction flask containing magnetic stirrer; B, gas buret; C, connection to deuterium cylinder; D, 3-way stopcock; E, hydrolysis flask with sealed on condenser; P, to Pirani gauge; V, to vacuum pumps.

reading of hydrogen- H_2^2 volume in the buret is taken after the reaction flask has warmed to room temperature. The reaction mixture (Note 2) is magnetically stirred and a gas pressure slightly above atmospheric is maintained by means of the mercury filled leveling bulb. According to the consumption of hydrogen- H_2^2 , the hydrogenation is complete in 7 hours (Note 3). After the reaction mixture is cooled in liquid nitrogen, the manifold and flasks A and E are evacuated and the product is vacuum-distilled into flask E.

(b) *Succinic- H_4^2 Acid.* The hydrolysis flask E with attached condenser is removed from the vacuum manifold. The ester is then hydrolyzed during 1 hour with 4 ml. of 10% sodium hydroxide solution heated to reflux. The basic solution is cooled and transferred into a continuous-type ether extraction apparatus, with the aid of 25 ml. of water. The resulting solution is acidified with dilute hydrochloric acid and extracted with ether for 48 hours. Removal of the ether leaves 120.7 mg. (96.7%) of colorless crystalline residue. The crude product is dissolved in dry acetone and transferred to a vacuum sublimator. The entire product, 120.2 mg. (96.2%), is sublimed during 6 hours at a bath temperature of $80-87^\circ$ and a pressure of $5.5-11 \times 10^{-3}$ mm.; m.p. $187-188^\circ$.

B. Notes

1. The catalyst was 5% palladium-on-carbon which was prepared free of adsorbed hydrogen by reduction of the palladium salt with formaldehyde.¹

2. Since the use of a solvent in the hydrogenation was unnecessary, the possibility of hydrogen exchange with the solvent was eliminated.

3. Practically pure hydrogen- H_2^2 was used in the hydrogenation. In a similar experiment using hydrogen under practically identical experimental conditions, the reaction was complete in 4.25 hours.

C. Other Preparations

Methyl succinate- H_4^2 has been prepared² by the deuteration of methyl acetylenedicarboxylate in ethyl acetate with a platinum catalyst. Succinic- H_4^2 acid was prepared from the ester by hydrolysis with dilute nitric acid.

Succinic- H_4^2 acid has also been obtained from succinic- H_4^2 acid- H_2^2 by exchange with water.³

Succinic acid- H_2^2 has been prepared by the hydrolysis⁴ of succinic anhydride with water- H_2^2 and by repeated recrystallization³ of succinic acid from water- H_2^2 .

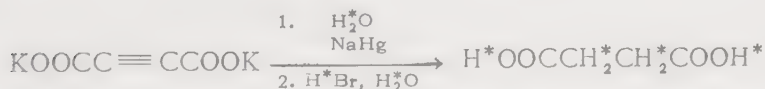
¹A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., New York, 1948, p. 990.

²A. McLean and R. Adams, *J. Am. Chem. Soc.*, 58, 804 (1936).

³J. O. Halford and L. C. Anderson, *ibid.*, 58, 736 (1936).

⁴J. M. Robertson and A. R. Ubbelohde, *Proc. Roy. Soc. (London)*, A170, 222 (1939).

SUCCINIC- H_4^2 ACID- H_2^2



J. O. Halford and L. C. Anderson, *J. Am. Chem. Soc.*, 58, 736 (1936).

A. Procedure

To 14 g. of potassium acetylenedicarboxylate (0.021 mole, Note 1), partially dissolved in 15 ml. of water- H_2^2 , is added, in portions with cooling and stirring, 2 g. of sodium dissolved in 20 ml. of mercury. The mixture is then stirred for 1 hour. The aqueous layer is separated from the mercury and acidified with 13.5 ml. of a 50% solution of hydrogen- H^2 bromide in water- H_2^2 (Note 2). The mixture is transferred to a 50-ml. flask in which crystallization and drying are effected by evaporation of the water- H_2^2 *in vacuo*; the latter is collected in a flask cooled with Dry Ice and acetone. The flask and its contents are crushed in a metal mortar and extracted with anhydrous ether in a Soxhlet apparatus. The ether is evaporated and replaced by benzene, which is used for filtering and washing the product. The yield is 2.2 g. (85%) (Note 3).

The product is dissolved in 2.5 ml. of warm water- H_2^2 , then recovered and dried by evaporation of the water *in vacuo* (Note 4). The final product melts at 178-179.1°.

B. Notes

1. Potassium acetylenedicarboxylate was prepared from dibromosuccinic acid as described by Ruggli.¹ It proved convenient to use the resulting mixture of potassium bromide and potassium acetylenedicarboxylate, since attempts to purify the latter were attended by polymerization and decomposition.

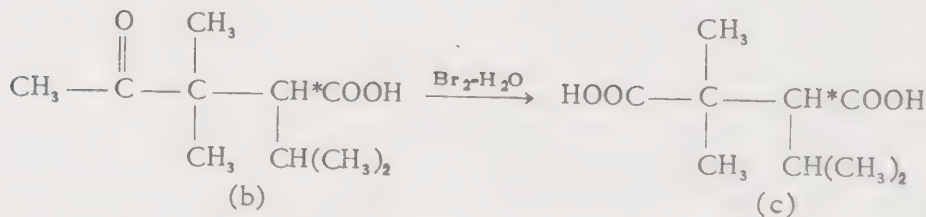
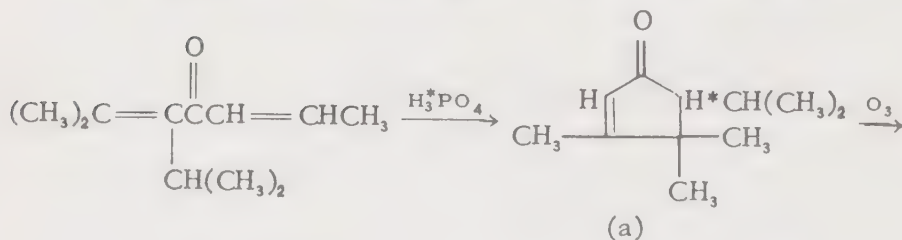
2. The solution was acidified to a deep blue on Congo red paper. The hydrogen- H^2 bromide solution, freshly prepared by dissolving liquid hydrogen- H^2 bromide in water- H_2^2 , was free from visible traces of bromine.

3. During the preparation, 28.5 ml. of water- H_2^2 was introduced and 28 ml. was recovered, the difference being approximately correct for the amount of deuterium present in succinic- H_4^2 acid- H_2^2 .

4. The last step was done to ensure the absence of hydrogen.

¹P. Ruggli, *Helv. Chim. Acta*, 3, 559 (1920).

2-ISOPROPYL-3,3-DIMETHYLSUCCINIC-2- H^2 ACID



D. N. Kursanov, Z. N. Parnes, I. I. Zaretskaya and I. N. Nazarov, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 1954, 859; through *Chem. Abstracts*, 49, 13910 (1955).

A. Procedure

(a) 5-Isopropyl-3,4,4-trimethyl-2-cyclopenten-1-one-5- H^2 . Upon treatment of 14.4 g. of 3-isopropyl-2-methyl-2,5-heptadien-4-one with 14.0 g.

of phosphoric acid- H_3^2 (Note 1) for 8 hours at 20–21°, 5-isopropyl-3,4,4-trimethyl-2-cyclopenten-1-one-5- H^2 is obtained (Note 2).

(b) *2-Isopropyl-3,3-dimethyllevulinic-2- H^2 Acid*. Treatment of the above cyclic ketone with ozone yields formic acid and higher organic acids from which 2-isopropyl-3,3-dimethyllevulinic-2- H^2 acid, m.p. 48°, is crystallized.

(c) *2-Isopropyl-3,3-dimethylsuccinic-2- H^2 Acid*. Oxidation of the above levulinic acid derivative with bromine water gives 2-isopropyl-3,3-dimethylsuccinic-2- H^2 acid, m.p. 134–135° (Note 3).

B. Notes

1. Phosphoric acid- H_3^2 was prepared from 9.6 g. of phosphorus pentoxide and 4.4 g. of water- H_2^2 .

2. 5-Isopropyl-3,4,4-trimethyl-2-cyclopenten-1-one, b.p. 112–112.5° (17 mm.), n_D^{20} 1.4779, d_{20} 0.9245, was prepared from 3-isopropyl-2-methyl-2,5-heptadien-4-one, b.p. 93–93.5° (12 mm.), n_D^{20} 1.4735, d_{20} 0.8799, by treatment of the latter with ordinary phosphoric acid.

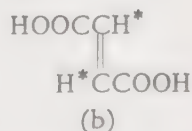
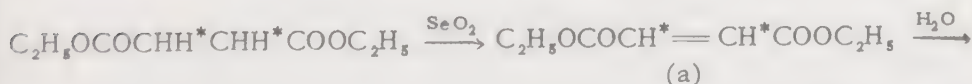
3. The succinic acid derivative retained 1 atom of deuterium per molecule, which was the amount present in the cyclic ketone.

C. Other Preparations

Nazarov, *et al.*,¹ have also cyclized 2-methyl-1,5-hexadien-3-one with phosphoric acid- H_3^2 , and obtained 2,4-dimethyl-2-cyclopenten-1-one-5- H_1^2 , which upon ozonolysis gave 2-methylsuccinic-3- H_1^2 acid. Oxidation of the cyclopentenone with selenium dioxide gave deuterium-free 3,5-dimethyl-3-cyclopentene-1,2-dione, m.p. 64–65°; hence the deuterium must have been attached to C-5.

¹I. N. Nazarov, I. I. Zaretskaya, Z. N. Parnes and D. N. Kursanov, *Izvest. Akad. Nauk S.S.S.R., Otdel. Khim. Nauk*, 1953, 519; through *Chem. Abstracts*, 48, 9930 (1954); *ibid.*, 1953, 114; through *Chem. Abstracts*, 48, 3271 (1954).

FUMARIC- H_2^2 ACID



H. Erlenmeyer, W. Schoenauer and H. Sullmann, *Helv. Chim. Acta*, 19, 1376 (1936).

A. Procedure

(a) *Ethyl Fumarate-H₂²*. Ethyl succinate-2,3-H₂² is dehydrogenated by adaptation of the procedure of Astin, Newman and Riley¹ (Note 1). A mixture of 8.7 g. of the ester and 11.2 g. of selenium dioxide is heated at 170° for 11 hours (Note 2). The ester (Note 3) is isolated and purified by vacuum distillation.

(b) *Fumaric-H₂² Acid*. The above ethyl fumarate-H₂² is hydrolyzed in alkaline solution. The reaction mixture is acidified with mineral acid, and the fumaric-H₂² acid is collected and recrystallized from water (Note 4).

B. Notes

1. In the selenium dioxide oxidation method¹ of preparing keto aldehydes and esters, ethyl succinate behaved abnormally, and the principal product was a mixture of ethyl fumarate and ethyl hydrogen fumarate in 40% yield.

2. The selenium dioxide is in excess of 2 moles to 1 of the ester; according to Astin,¹ the maximum yield of ethyl fumarate is obtained under these conditions. Even when an excess of ethyl succinate is used, the main product is ethyl fumarate.

3. According to Astin,¹ the product is a mixture; see Note 1. The product was first extracted with ether and then fractionally distilled in vacuum. After a forerun of 3 ml. with b.p. up to 85° (30 mm.), a 35-g. fraction boiling up to 150° (30 mm.) was collected. When refractionated, most of this material was the diethyl ester, b.p. 110–112° (22 mm.), with 3.05 g. of ethyl hydrogen fumarate, b.p. 138° (22 mm.). In the initial fractionation, 17.4 g. of ethyl hydrogen fumarate, m.p. 60–63°, was collected at 150° (30 mm.).

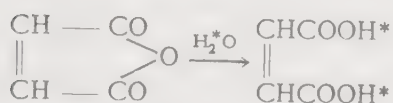
4. According to the isotopic analyses, the molecular formula of the product was C₂H_{0.96}H_{1.04}²(COOH)₂, and that of the initial ethyl succinate-2,3-H₂² was C₂H_{2.02}H_{1.98}²(COOC₂H₅)₂. It was then calculated that the ratio of hydrogen to deuterium in the hydrogen removed from the ester by dehydrogenation would be:

$$\frac{1}{4}(\text{H}_{2.02}\text{H}_{1.98}^2) = \text{H}_{0.505}\text{H}_{0.495}^2$$

and according to the analysis of the product the value would be:

$$\frac{1}{2}(\text{H}_{2.02}\text{H}_{1.98}^2 - \text{H}_{0.96}\text{H}_{1.04}^2) = \text{H}_{0.53}\text{H}_{0.47}^2$$

¹S. Astin, A. C. C. Newman and H. L. Riley, J. Chem. Soc., 1933, 391.

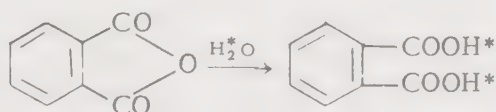
MALEIC ACID- H_2^2 

H. M. E. Cardwell, J. D. Dunitz and L. E. Orgel, J. Chem. Soc., 1953, 3740.

Procedure

(a) *Maleic Acid- H_2^2* . Maleic anhydride is dissolved in warm water- H_2^2 , and the solution is evaporated at room temperature *in vacuo*.

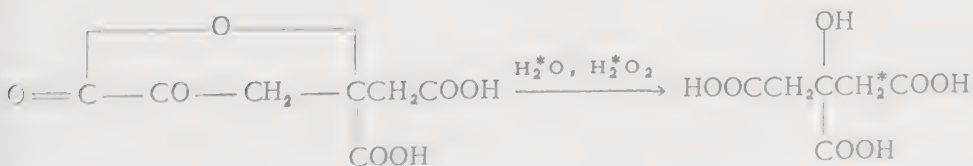
(b) *Potassium Hydrogen- H^2 Maleate*. Anhydrous potassium hydrogen maleate is dissolved in an excess of water- H_2^2 . The water- H_2^2 is removed by distillation, and the salt is dried *in vacuo*.

PHTHALIC ACID- H_2^2 

J. M. Robertson and A. R. Ubbelohde, Proc. Roy. Soc. (London), A170, 222 (1939).

Procedure

Phthalic anhydride, 1 g., is heated under reflux with 3 g. of water- H_2^2 (99.6%) for 2 hours with atmospheric moisture excluded by a guard tube. The mother liquor is removed by decantation from the crystals obtained upon cooling the solution, and the product is recrystallized from hot water- H_2^2 ; m.p. 191.0° with loss of water. The ordinary acid, prepared in the same manner, melts at 193° with loss of water.

CITRIC-2- H_2^2 ACID

C. Martius and G. Schorre, Ann., 570, 140 (1950); Z. Naturforsch., 5b, 170 (1950).

A. Procedure

(a) *DL-Citric-2-H₂ Acid*. Oxalcitramalic acid,¹ 1.5 g., is dissolved in 1.5 ml. of water-H₂ (96.3%), and after 12 hours the water-H₂ is distilled off *in vacuo*. This process is repeated four times. The final residue is again dissolved in a little water-H₂, 0.9 ml. of hydrogen-H₂ peroxide (Note 1) is added, and the mixture is kept for 24 hours. The hydrogen-H₂ peroxide and water-H₂ are removed *in vacuo*, and the residue is successively treated with ordinary water and evaporated to dryness 5 times (Note 2). After recrystallization from ethyl acetate-petroleum ether, the colorless crystals weigh 810 mg. and melt at 152–153° (Note 3).

(b) *(-)-Citric-2-H₂ Acid*. (-)-Oxalcitramalic acid, 1.23 g., is treated 5 times with 5-ml. amounts of water-H₂ and then oxidized with 4 ml. of 10.2% hydrogen-H₂ peroxide, as described above (Note 4).

The yield of L-citric-2-H₂ acid, m.p. 148–149° after recrystallization from acetic acid-petroleum ether, is 420 mg. (Note 5).

(c) *(+)-Citric-2-H₂ Acid*. The procedure is the same as that described above. The yield of the D-acid is 760 mg. (55%); m.p. 149–150.5° (Note 6).

B. Notes

1. The preparation of 29% hydrogen-H₂ peroxide is described by Martius and Schorre.

2. The exchangeable hydrogen-H² atoms are removed from the carboxyl and hydroxyl groups in this manner.

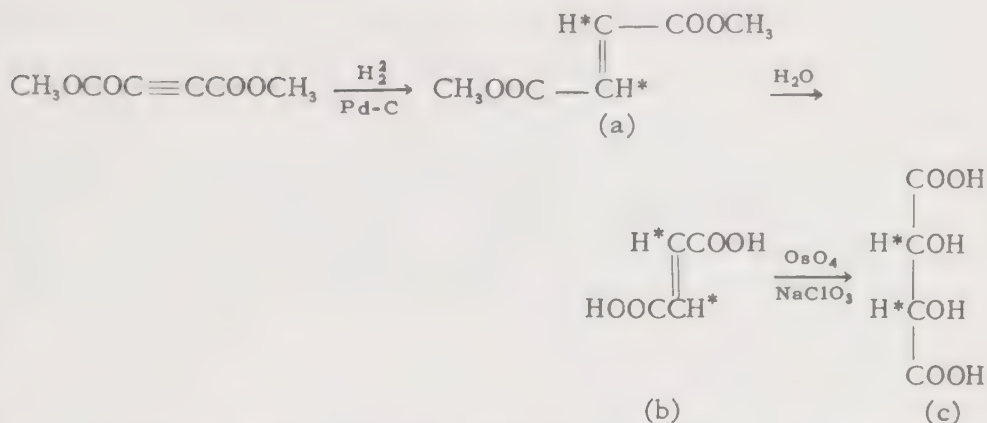
3. According to the isotopic analysis, the deuterium content of the product corresponded to 79.4% exchange of the hydrogen atoms of one methylene group.

4. The resolution of DL-oxalcitramalic acid by means of the brucine salt is described by Martius and Schorre.

5. The specific rotation of the product was $[\alpha]_{546}^{20} - 33.6 \pm 2^\circ$ in saturated ammonium molybdate solution, and analysis indicated 96.1% exchange of the hydrogen atoms of one methylene group.

6. The rotation in pure water (c, 12.61 g./100 ml.) was $[\alpha]_{546}^{20} + 1.03 \pm 0.06^\circ$; in ammonium molybdate solution, $[\alpha]_{546}^{20} = + 31.9 \pm 2^\circ$ (c, 2.730 g./100 ml.). The isotopic analysis indicated 95.9% exchange of the hydrogen atoms of one methylene group.

¹C. Martius, Z. physiol. Chem., 279, 102 (1943).

TARTARIC-2,3- H_2^2 ACID

H. Erlenmeyer, O. Bitterlin and H. M. Weber, *Helv. Chim. Acta*, 22, 701 (1939).

A. Procedure

(a) *Methyl Fumarate- H_2^2* . The half-hydrogenation of acetylenedicarboxylic acid is done essentially according to the procedure of Ott and Schröter¹ (Note 1). Methyl acetylenedicarboxylate, 10 g., is dissolved in 10 ml. of ethyl acetate and shaken with hydrogen- H_2^2 (99.6%) in the presence of palladium-on-carbon catalyst. The calculated amount of hydrogen- H_2^2 is taken up after 9 hours. Since a mixture of maleic and fumaric acid esters is formed in the reduction, the former is converted to the latter by the procedure of Anschütz.² After removal of the catalyst and solvent, the mixture of esters is heated under reflux for several hours with a trace of iodine and then fractionally distilled. After further purification by recrystallization and sublimation under vacuum, the methyl fumarate- H_2^2 melts at 105.5°.

(b) *Fumaric- H_2^2 Acid*. The methyl fumarate- H_2^2 , 4 g., is heated under reflux for several hours with a solution of 5.5 ml. of 0.2 N nitric acid in 40 ml. of water. Fumaric- H_2^2 acid crystallizes from the cooled solution.

(c) *Tartaric-2,3- H_2^2 Acid*. To a solution of 4.8 g. of sodium chlorate in 40 ml. of water is added 3.5 g. of fumaric- H_2^2 acid. After the acid is neutralized with sodium carbonate, 0.015 g. of osmium tetroxide is added, and the solution is warmed for 48 hours at 40-45°.

The solution is filtered, and the tartaric-2,3- H_2^2 acid is precipitated as the lead salt by adding lead acetate. The lead salt is collected, washed with water and dissolved in ammonium hydroxide solution. The solution is filtered, warmed to remove excess ammonia and treated with acetic acid to again precipitate the lead salt of tartaric-2,3- H_2^2 acid. The salt is then suspended in water and treated with hydrogen sulfide until no more lead sulfide precipitates. The clear filtrate, free of lead sulfide,

is concentrated to obtain crystalline tartaric-2,3- H_2^2 acid. After recrystallization from absolute alcohol (Note 2), the product melts at 204–205° (Note 3).

B. Notes

1. Ott and Schröter¹ reduced ethyl acetylenedicarboxylate in ethanol in the presence of a palladium catalyst with excellent results. Erlenmeyer, *et al.*, suspected that exchange of hydrogen- H_2^2 with the hydrogen of methanol would occur. A trial experiment proved this to be the case.

2. Tartaric acid is recrystallized from absolute alcohol without water of crystallization.³

3. From a portion of the acid, the more difficulty soluble ammonium hydrogen tartrate-2,3- H_2^2 was formed by the careful addition of ammonia to an aqueous solution of the acid. Isotopic analysis of the acid and the salt gave the following molecular formulae, respectively: $C_4H_{4.05}H_{1.95}O_6$ and $C_4H_{4.1}H_{1.9}O_6$.

C. Other Preparations

Fumaric acid- H_2^2 has been prepared⁴ by heating maleic anhydride at 130–170° in a sealed tube with water- H_2^2 .

Crystalline sodium ammonium tartrate-2,3- H_2^2 has been prepared⁵ according to the procedure of Pasteur.⁶

¹E. Ott and R. Schröter, *Ber.*, 60, 624 (1927).

²R. Anschütz, *Ber.*, 12, 2283 (1879).

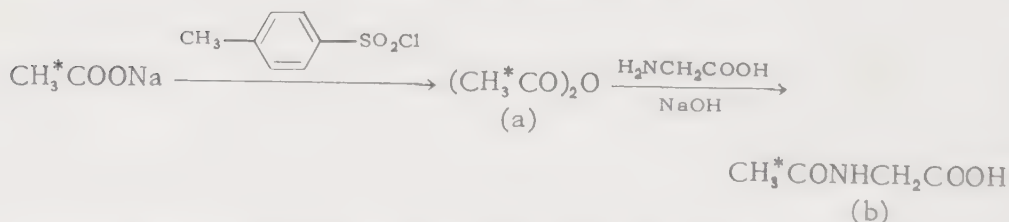
³W. H. Perkin, *J. Chem. Soc.*, 1887, 362.

⁴A. R. Ubbelohde, *Proc. Roy. Soc. (London)*, A173, 417 (1939).

⁵H. Erlenmeyer and O. Bitterlin, *Helv. Chim. Acta*, 23, 207 (1940).

⁶M. L. Pasteur, *Ann. Chim.*, (3), 24, 442 (1848); *ibid.*, 28, 56 (1850).

N-ACETYL- H_3^3 -GLYCINE



K. Bloch and D. Rittenberg, *J. Biol. Chem.*, 169, 467 (1947).

A. Procedure (Note 1)

(a) *Bis(acetic- H_3^3) Anhydride.* A mixture of 0.1 mole of anhydrous sodium acetate- H_3^3 with 0.1 mole of *p*-toluenesulfonyl chloride is heated at

220°. ¹ The bis(acetic-H₃²) anhydride which distills is collected in an ice-cooled receiver.

(b) *N*-Acetyl-H₃²-glycine. The amino acid is dissolved in 2 equivalents of sodium hydroxide solution. This solution is cooled in an ice-bath, and 1 equivalent each of bis(acetic-H₃²) anhydride and of sodium hydroxide solution are added gradually with stirring. The stirring is continued for 20 minutes, when the solution is filtered and acidified with dilute sulfuric acid. The *N*-acetyl-H₃²-glycine is recrystallized from water. The *N*-acetyl-H₃²-amino acids prepared by this method are listed in Table XVI, 2.

TABLE XVI, 2
N-Acetyl-H₃²-Amino Acids

Compound	Recrystallization solvent	m.p., °C.	[α] _D
<i>N</i> -Acetyl-glycine	water	208-209
<i>N</i> -Acetyl-L-leucine	water	189-190	-22.5° (2% in absolute alcohol)
<i>N</i> -Acetyl-D-leucine	water	190	+24° (1% in absolute alcohol)
<i>N</i> -Acetyl-L-glutamic acid	water	201	+4.7° (2% in <i>N</i> sodium hydroxide)
<i>N</i> -Acetyl-L-alanine	ethyl acetate	130-132	-60.2° (1% in water)
<i>N</i> -Acetyl-D-alanine	ethyl acetate	131-132	+63.4° (1% in water)
<i>N</i> -Acetylsarcosine	acetone	138
<i>N</i> ⁶ -Acetyl-L-lysine*	water	249-250°	+4.0° (4% in water)

*Note 2

B. Notes

1. This is a general procedure for the acetylation of amino acids.

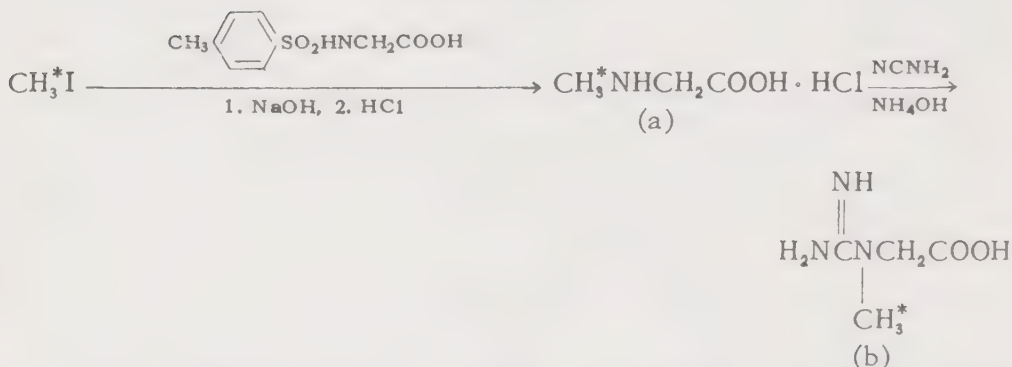
2. *N*⁶-Acetyl-L-lysine was prepared by the method of Neuberger and Sanger.² Excess (basic) copper carbonate is added gradually to a boiling solution of L-lysine sulfate (1.0 g.). After cooling, the solution is filtered, 1 equivalent of barium hydroxide (1.7 g.) is added, and the solution is cooled in ice. Then 1.2 g. of acetic anhydride and 2.2 g. of barium hydroxide are added in portions during 30 minutes with shaking and cooling. After the solution stands for 20 minutes at room temperature, 0.34 ml. (0.63 g.) of sulfuric acid is added, and hydrogen sulfide is passed through the solution. The barium sulfate and copper sulfide are filtered off and washed well with hot water. The combined filtrate and washings are concentrated to 20 ml., and any excess of barium or sulfate ions is removed. The solution is evaporated to dryness, the residue is dissolved in a minimum of hot water, and absolute ethanol is added to incipient cloudiness. On cooling the solution, the *N*⁵-acetyl-lysine crystallizes in flat plates, which are collected and washed with

aqueous ethanol, absolute ethanol and ether. The yield is 0.6 g. Another 0.4 g. may be obtained from the mother liquor.

¹German patent 123,052; Chem. Zentr., 2, 518 (1901).

²A. Neuberger and F. Sanger, Biochem. J., 37, 515 (1943).

N-AMIDINO-N-METHYL- H_3^2 -GLYCINE
(H_3^2 -Creatine)



M. Cohn, S. Simmonds, J. P. Chandler and V. du Vigneaud, J. Biol. Chem., 162, 343 (1946).

A. Procedure

(a) *N-Methyl- H_3^2 -glycine Hydrochloride*, (H_3^2 -Sarcosine Hydrochloride). H_3^2 -Sarcosine hydrochloride is prepared from methyl- H_3^2 iodide and *N-p*-toluenesulfonylglycine according to the following procedure described by Fischer and Bergmann.¹ A mixture of 38 g. of *N-p*-toluenesulfonylglycine, dissolved in 200 ml. of 3 *N* sodium hydroxide solution, and 28 g. of methyl iodide, in a stoppered flask, is shaken in a bath at 67°. After about 10 minutes, a clear solution results which is kept at 67° for another 50 minutes. The solution is then cooled and acidified. The oil which separates soon crystallizes when cooled in ice. The crude product is dissolved in potassium carbonate solution, precipitated by the addition of hydrochloric acid and recrystallized from 1 l. of hot water. The yield of *N-p*-toluenesulfonylsarcosine, m.p. 150–152° (cor.), is 38 g. This compound is soluble in acetone and alcohol and slightly soluble in benzene and petroleum ether.

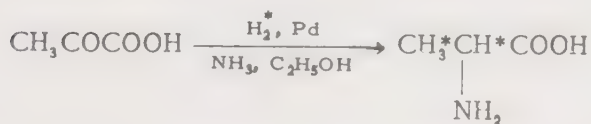
To obtain sarcosine, 10 g. of the *p*-toluenesulfonyl derivative is heated in a sealed tube at 100° with 40 ml. of hydrochloric acid (d 1.19) for 22 hours. When the solution is cooled to 0°, *p*-toluenesulfonic acid crystallizes and is filtered off. The filtrate is evaporated, leaving a crystalline residue, which is dissolved in 20 ml. of alcohol and recrystallized by the

addition of ether. The yield of crystalline sarcosine hydrochloride, m.p. 171–174°(cor.), is 4.7 g. (91%).

(b) *N-Amidino-N-methyl-H₃²-glycine, (H₃²-Creatine)*. A solution of 4.4 g. of H₃²-sarcosine hydrochloride, dissolved in 14 ml. of water, is treated with 4 ml. of concentrated ammonium hydroxide and 2.8 g. of cyanamide in 10 ml. of water. After 3 days at room temperature, the H₃²-creatine hydrate is collected, recrystallized from water and dried to constant weight in an oven at 100°. The anhydrous H₃²-creatine obtained weighs 2.84 g.

¹E. Fischer and M. Bergmann, *Ann.*, 398, 96 (1913).

ALANINE-2,3-H₄²



K. Bloch and D. Rittenberg, *J. Biol. Chem.*, 159, 45 (1945).

A. Procedure

Alanine-2,3-H₄² is prepared by the reduction of pyruvic acid with hydrogen-H₂² and a palladium-black catalyst in an ethanolic solution of ammonia; see glutamic-2,3-H₃² acid and alanine-N¹⁵.

B. Other Preparations

Bloch and Rittenberg have also prepared: alanine-2,3-H₂², by the bromination of propionic-2,3-H₂² acid and reaction of the resulting 2-bromopropionic-2,3-H₂² acid with ammonia; and alanine-2,3-H₄² from acetaldehyde-H₄² and ammonium cyanide by the Strecker cyanohydrin synthesis.

B. Notes

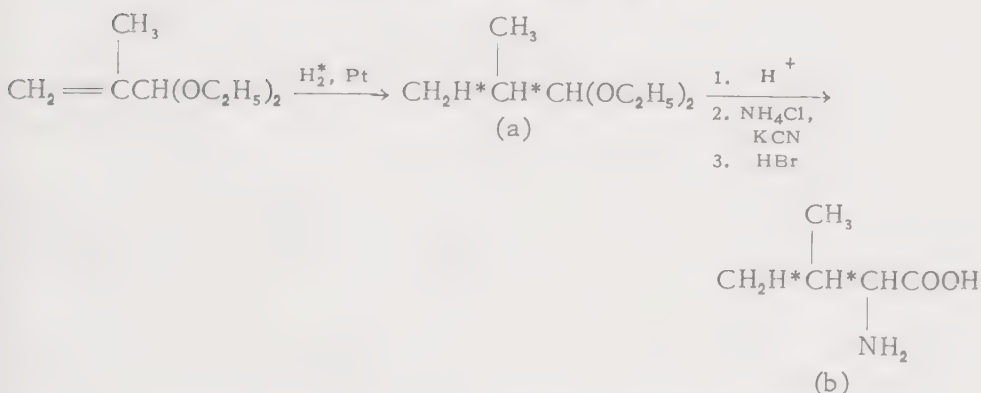
1. Considerable exchange took place with the hydrogen of the solvent, and only 15% of the deuterium present in the ethyl formate was left in the ethyl α -formylhippurate.

2. Rat and hog kidney possess an enzyme which acts rapidly and asymmetrically upon *N*-acylated amino acids, hydrolyzing the acyl radical completely from the L-form and leaving the *N*-acylated D-form intact. The chloroacetyl derivative of alanine was the most susceptible to hydrolysis of the acyl derivatives studied and was hydrolyzed roughly 3-4 times more rapidly than the corresponding acetyl derivative.

¹V. E. Price, J. B. Gilbert and J. P. Greenstein, *J. Biol. Chem.*, 179, 1169 (1949).

²E. Fischer and H. Roesner, *Ann. Chem.*, 375, 199 (1910).

VALINE-3,4-H₂²
(2-Amino-3-methylbutyric-3,4-H₂² Acid)



C. R. Kinney and R. Adams, *J. Am. Chem. Soc.*, 59, 897 (1937).

A. Procedure

(a) *2-Methylpropionaldehyde-2,3-H₂² Ethyl Acetal*. Methacrylaldehyde ethyl acetal (Note 1) dissolved in ethyl acetate is hydrogenated with hydrogen-H₂² and platinum catalyst¹ (Note 2). After removal of the catalyst, the pure product, b.p. 133-135° (747 mm.), is obtained by fractionation of the ethyl acetate solution; d_4^{20} 0.8368; n_D^{20} 1.3938.

(b) *Valine-3,4-H₂², (2-Amino-3-methylbutyric-3,4-H₂² Acid)*. This compound is prepared according to the procedure described for leucine-3,4-H₂². The yield of valine-3,4-H₂² is 12.2 g. (43.4%). After recrystallization from water, using activated carbon to remove a faint yellow color, the product melts at 273° with decomposition (Note 3).

B. Notes

1. Methacrylaldehyde ethyl acetal is obtained according to the procedures of Fischer² and Dworzak and Prodinger.³

2. Details of the equipment and procedure used by Adams and co-workers are given in earlier publications.^{4,5}

3. Analysis of the product for deuterium indicated that the product contained about 25% less deuterium than the calculated amount. This difference was probably due to the replacement of deuterium by hydrogen during the transformation of the 2-methylpropionaldehyde-2,3-H₂² ethyl acetal through the aldehyde to the amino acid. The tertiary hydrogen-H² atom alpha to the aldehyde group is subject to enolization and exchange.

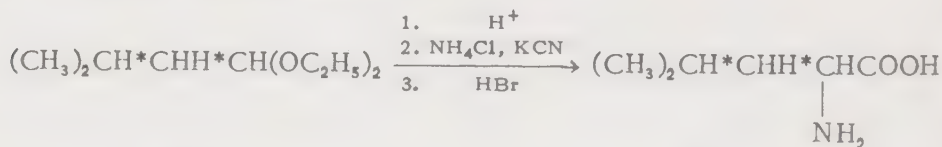
¹*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1941, p. 463.

²F. G. Fischer, L. Ertel and K. Loewenberg, *Ber.*, 64B, 30 (1931).

³R. Dworzak and W. Prodinger, *Monatsh.*, 53, 590 (1929).

⁴M. T. Leffler and R. Adams, *J. Am. Chem. Soc.*, 58, 1555 (1936).

⁵A. McLean and R. Adams, *ibid.*, 58, 804 (1936).

LEUCINE-3,4-H₂²(2-Amino-4-methylvaleric-3,4-H₂² Acid)

(a)

(b)

C. R. Kinney and R. Adams, *J. Am. Chem. Soc.*, 59, 897 (1938).

A. Procedure

(a) 3-Methylbutyraldehyde-2,3-H₂² Ethyl Acetal. Senecialdehyde ethyl acetal (Note 1), dissolved in dioxane (Note 2), is hydrogenated with hydrogen-H₂² and Raney nickel catalyst. After removal of the catalyst, fractionation of the dioxane solution yields a product with b.p. 164-165° (740 mm.); n_D^{20} 1.4025; d_4^{20} 0.8423 (Note 3).

(b) Leucine-3,4-H₂², (2-Amino-4-methylvaleric-3,4-H₂² Acid). A mixture of 35 g. of 3-methylbutyraldehyde-2,3-H₂² ethyl acetal (Note 4), 125 ml. of water and 15 drops of concentrated sulfuric acid is stirred for 30 minutes. The free 3-methylbutyraldehyde-2,3-H₂² is distilled slowly into a flask which is cooled in ice, and contains 100 ml. of methanol, 35 g. of ammonium chloride and 35 g. of potassium cyanide. When all the aldehyde has distilled, the solution is refluxed for 2 hours. After cooling the

solution, 100 ml. of ether is added, and the precipitated salt is filtered off and washed with ether. To the alcohol-ether solution is added 100 ml. of 40% hydrobromic acid and 25 ml. of water. After the mixture stands for 2 hours, it is heated under an air condenser until the alcohol and ether are removed. Water is added to replace that lost by evaporation, and the mixture is refluxed for 12 hours, using a water-cooled condenser.

The solution is evaporated to dryness, finally, under reduced pressure. The solid residue is dissolved in about 200 ml. of water, the solution is cooled in ice, and well-washed silver oxide (Note 5) is added in small lots. When an excess of silver oxide is present, the solution is filtered, and the precipitate is washed well with water. Hydrogen sulfide is passed into the solution until silver sulfide precipitation is complete. The filtered solution is concentrated until the amino acid begins to crystallize. Methyl alcohol is added, and the solution is cooled in ice. The product is recrystallized from water solution which is decolorized with activated carbon. The yield of leucine-3,4-H₂² is about 31%, m.p. 271° (dec., sealed tube).

B. Notes

1. Senecialdehyde ethyl acetal is prepared¹ from isovaleraldehyde made from synthetic isoamyl alcohol.

2. Some difficulty was encountered in the reduction of the acetal with hydrogen-H₂². The best results were obtained with dioxane (distilled from sodium) as solvent, freshly distilled acetal and Raney nickel.

3. The calculated² value for the density of 2-methylbutyraldehyde-2,3-H₂² ethyl acetal, on the assumption of the same molecular volume for hydrogen-H₂² and hydrogen, is 0.8464 as compared with this found value of 0.8423.

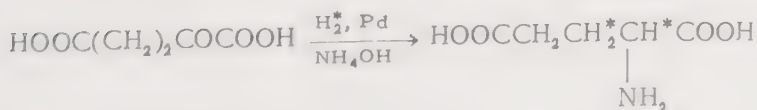
4. This material did not react with potassium permanganate solution in 3-4 minutes.

5. The silver oxide is prepared from silver nitrate.

¹F. G. Fischer, L. Ertel and K. Loewenberg, *Ber.*, 64B, 30 (1931); R. Dworzak and W. Prodinger, *Monatsh.*, 53, 588 (1929).

²A. McLean and R. Adams, *J. Am. Chem. Soc.*, 58, 804 (1936).

GLUTAMIC-2,3-H₂² ACID



D. Rittenberg, S. Ratner and H. D. Hoberman, *J. Am. Chem. Soc.*, 62, 2249 (1940).

A. Procedure

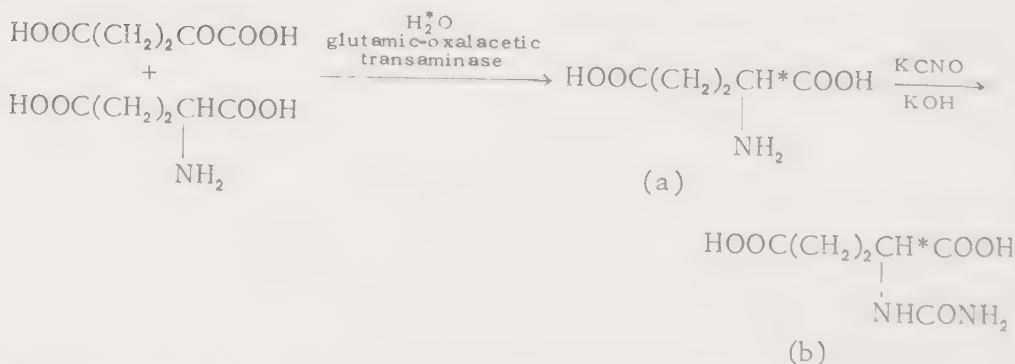
2-Oxoglutaric acid, 0.020 mole, is dissolved in 2.25 moles of water containing 0.060 mole of ammonia. This solution is shaken for 6 hours with palladium catalyst in an atmosphere of hydrogen- H_2^2 (Note 1). The resulting glutamic-2,3- H_3^2 acid contains 15.4 atom per cent deuterium (Note 2).

B. Notes

1. When 2-oxoglutaric acid was reduced with ordinary hydrogen in an ammoniacal solution of water- H_2^2 (6.7 atom per cent) with palladium catalyst, the glutamic acid formed contained only 0.41 atom per cent deuterium.

2. On refluxing this H^2 -glutamic acid in normal water with 20% hydrochloric acid for 5 days, no change in deuterium concentration occurred. The barium succinate obtained by degradation of the glutamic acid had the formula $C_4H_{2.87}H_{1.13}O_4$ Ba. From these figures it was calculated, assuming no loss of deuterium during the degradation, that the 2-hydrogen atom contained 26 atom per cent, and the 3-hydrogen atoms contained 56 atom per cent, of deuterium. This is not as would be expected, since the keto-enol mechanism would tend to introduce normal hydrogen from the water into the 3-position. The 3-hydrogen atoms of the dissolved 2-oxoglutaric acid appear to have exchanged with hydrogen- H_2^2 of the gas phase. According to Ratner, Rittenberg and Schoenheimer,¹ the deuterium atoms at the 2- and 3-positions of glutamic acid are stable towards acid, but those at the 4-position are semilabile.

¹S. Ratner, D. Rittenberg and R. Schoenheimer, J. Biol. Chem., 135, 357 (1940).

N-CARBAMOYL-L-GLUTAMIC-2- H^2 ACID

S. Grisolia and R. H. Burris, J. Biol. Chem., 210, 109 (1954).

A. Procedure

(a) *L-Glutamic-2-H² Acid*. To 176 g. of water-H₂, adjusted to pH 7.2 with solid sodium hydroxide, is added 0.90 g. of glutamic acid, 0.096 g. of 2-oxoglutaric acid and 50 mg. of purified glutamic-oxalacetic transaminase (Note 1). After the mixture is incubated for 6 hours at 38°, 2 g. of solid trichloroacetic acid is added. The mixture is frozen and concentrated to about 40 ml. by lyophilization. Then, 100 ml. of 10% perchloric acid and 20 ml. of water are added, and the mixture is stored for 6 hours in the cold. The denatured protein is filtered off, and the filtrate is extracted with 200 ml. of ether. The ether phase is separated and extracted with 20 ml. of water, which is then combined with the main water phase. To this solution is added 5 g. of barium perchlorate and sufficient solid sodium hydroxide to make the solution basic to phenolphthalein indicator. After 1 hour, 800 ml. of 95% ethanol is added, and the mixture is refrigerated for 12 hours. The precipitate is collected by centrifugation and dissolved in 100 ml. of water. The barium-alcohol precipitation procedure is repeated twice. Finally the precipitate is dissolved in about 60 ml. of water, barium is quantitatively removed by addition of potassium sulfate, and the water phase is carefully concentrated to dryness, at room temperature, in a desiccator over calcium chloride.

The dry material is dissolved in 6 ml. of water, in several portions; the solution is heated to 60° and filtered. The filtrate is adjusted to pH 3.2, first with concentrated and then with 2 *N* hydrochloric acid. The precipitate, which forms when the solution is cooled, is collected, washed successively with 1 ml. of cold water and with alcohol, and dried. The yield of *L*-glutamic-2-H² acid is 762 mg. (Note 2).

(b) *N-Carbamoyl-L-glutamic-2-H² Acid*. A mixture of 0.650 g. of glutamic-2-H² acid, 0.358 g. of potassium cyanate and 4.4 ml. of 1 *N* potassium hydroxide is kept at room temperature for 16 hours (Note 3). The solution is acidified with concentrated hydrochloric acid, and after 2 hours the product is collected.

B. Notes

1. The enzyme was obtained from heart muscle according to the procedure of Cammarata and Cohen;¹ 1 mg. of enzyme was equal to 5000 units of activity.²

2. The calculated deuterium excess for total exchange in the 2-position was 11.1%; that found was 11.2%.

3. This is an adaptation of the procedure of Nyc and Mitchell³ for the preparation of *N*-carbamoylaspartic acid.

C. Other Preparations

L-Glutamic-2- H^2 acid has been prepared by enzymatic methods.^{4,5}

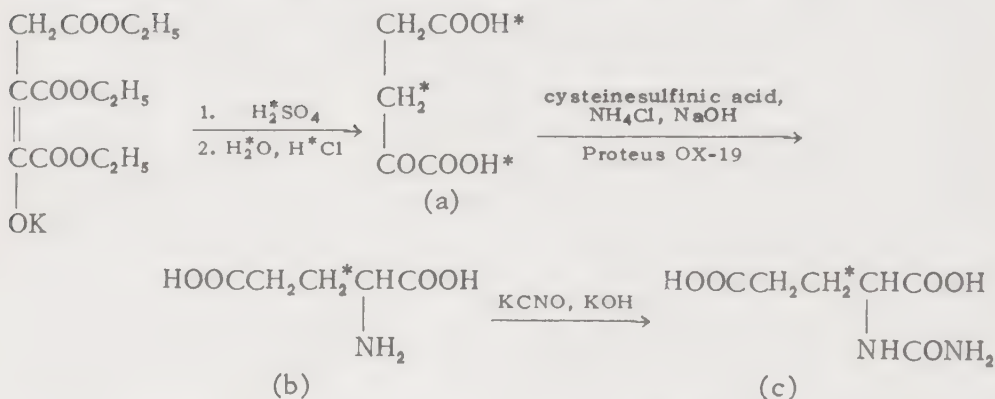
¹P. S. Cammarata and P. P. Cohen, *J. Biol. Chem.*, **193**, 53 (1951).

²*Idem*, **193**, 45 (1951).

³J. F. Nyc and H. K. Mitchell, *J. Am. Chem. Soc.*, **69**, 1382 (1947).

⁴A. S. Konikova, N. N. Dobbelt and A. E. Braunstein, *Nature*, **159**, 67 (1947).

⁵D. B. Sprinson and D. Rittenberg, private communication to P. P. Cohen.

N-CARBAMOYL-L-GLUTAMIC-3- H^2 ACID

S. Grisolia and R. H. Burris, *J. Biol. Chem.*, **210**, 109 (1954).

A. Procedure

(a) 2-Oxoglutaric-3- H^2 Acid- H^2 . A solution of 31.5 g. of ethyl potassiumoxalosuccinate¹ in 45 ml. of water- H^2 is extracted twice with 50-ml. portions of ether. The aqueous solution is then acidified to pH 2.0 with about 3 *N* sulfuric acid- H^2 (Note 1). This mixture is extracted six times with 50-ml. portions of dry ether. The ether extracts are combined, dried over sodium sulfate, filtered and evaporated to dryness under reduced pressure. To effect hydrolysis the oxalosuccinic ester is refluxed with 160 ml. of 4.3 *N* hydrochloric acid- H^2 for 8–10 hours (Note 2). Then, the solution is evaporated to dryness under reduced pressure, and the solid residue is recrystallized from ethyl acetate. The yield of 2-oxoglutaric-3- H^2 acid- H^2 , m.p. 110° , is 10 g.

(b) L-Glutamic-3- H^2 Acid. A solution of 30 mmoles of 2-oxoglutaric-3- H^2 acid- H^2 , 30 mmoles of cysteinesulfinic acid and 6 mmoles of manganous chloride is adjusted to pH 9.4 with concentrated sodium hydroxide and then diluted to 450 ml. After 150 ml. of 0.5 *M* ammonium chloride-sodium hydroxide buffer, pH 9.4, is added, the mixture is completed with 2 g. of a lyophilized preparation of *Proteus* OX-19. The mixture, divided into 2 aliquots in two 1-l. flasks, is shaken at 35° for 1 hour. At this

time, the mixture is deproteinized by heating it in a boiling water-bath for 5 minutes. The mixture is cooled and filtered, and the filtrate is treated with 47 g. of barium perchlorate trihydrate. The precipitate is removed by filtration; the clear filtrate is treated with 4 volumes of 95% ethanol and refrigerated for 5 hours. The resulting precipitate is collected by centrifugation and dissolved in 300 ml. of water. This solution is taken to dryness by lyophilization, and the residue is extracted with about 25 ml. of water heated to 60°. After the solution is cooled and filtered, the filtrate is adjusted to pH 3.2 with concentrated hydrochloric acid. The solution is cooled, and the crystalline product is recrystallized from water; the yield of L-glutamic-3-H₂² acid is 1.27 g. (Note 3).

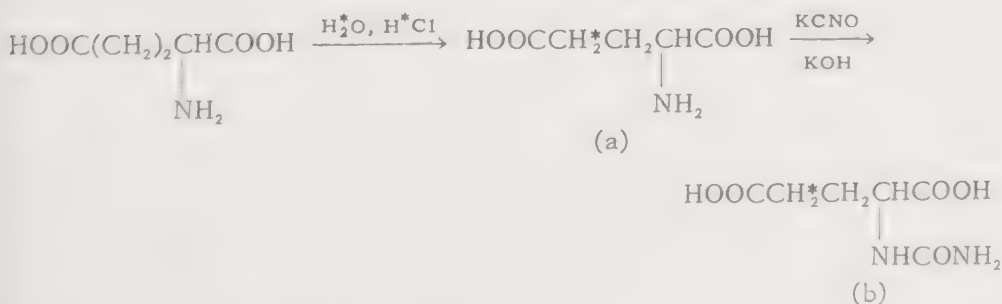
(c) *N*-Carbamoyl-L-glutamic-3-H₂² Acid. The *N*-carbamoyl derivative of L-glutamic-3-H₂² acid is prepared according to the procedure described for *N*-carbamoyl-L-glutamic-2-H² acid.

B. Notes

1. This is prepared by the addition of 3 ml. of fuming sulfuric acid to 34 ml. of water-H₂².
2. To 115 ml. of water-H₂² is added 60 ml. of concentrated hydrochloric acid.
3. There was about 26% loss of deuterium in the conversion of 2-oxoglutaric-3-H₂² acid to glutamic-3-H₂² acid. It was shown by experiment that this loss was not the result of the transamination reaction but, rather, was the result of hydrogen exchange at pH above 7. At pH 13.0 and a temperature of 38°, the exchange of hydrogen for deuterium in the 2-oxoglutaric-3-H₂² acid was practically quantitative in 30 minutes. The reverse exchange then affords a much simpler means of labeling this compound.

¹W. Wislicenus and M. Waldmüller, *Ber.*, 44, 1564 (1911).

N-CARBAMOYL-L-GLUTAMIC-4-H₂² ACID



S. Grisolia and R. H. Burris, *J. Biol. Chem.* 210, 109 (1954).

A. Procedure

(a) *L-Glutamic-4-H₂² Acid*. A suspension of 0.05 mole of *L*-glutamic acid in 52 ml. of 6 *N* hydrochloric acid-H² is heated in a sealed tube at 100° for 5 days (Note 1). The product is recrystallized from water to remove exchangeable deuterium from the amino and carboxyl groups.

(b) *N-Carbamoyl-L-glutamic-4-H₂² Acid*. The *N*-carbamoyl derivative is prepared according to the procedure for *N*-carbamoyl-*L*-glutamic-2-H² acid.

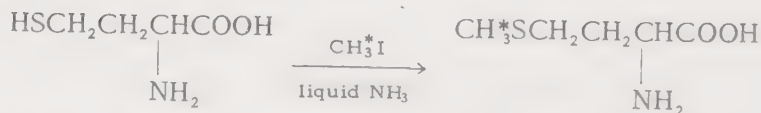
B. Notes

1. It has been shown that glutamic acid has some stably bound hydrogen¹ and some that is exchanged at a very slow rate when glutamic acid is heated with 20% hydrochloric acid-H².² The latter reaction is half completed at 100° in about 4 days. This deuterium is not in the 2-position, since introduction of deuterium does not parallel racemization, and the succinic acid obtained by oxidation of the glutamic acid contains all the deuterium originally present. It was shown² that the glutamic acid prepared by the catalytic hydrogenation of 2-oxoglutaric acid with hydrogen-H₂², in the presence of ammonia, has deuterium in the 2- and 3-positions which is not removed during prolonged boiling with 20% hydrochloric acid. Therefore, the semilabile deuterium, which is introduced by exchange in acid medium, must be in the 4-position.

¹G. L. Foster, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **125**, 13 (1938).

²S. Ratner, D. Rittenberg and R. Schoenheimer, *ibid.*, **135**, 357 (1940).

2-AMINO-4-(METHYLTHIO-H₃²)BUTYRIC ACID
(H₃²-Methionine)



V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, *J. Biol. Chem.*, **140**, 625 (1941).

Procedure

Methyl-H₃² iodide is used for the methylation of homocysteine in liquid ammonia according to the following procedure of du Vigneaud, Dyer and Harmon.¹ Homocysteine,² 0.5 g., is dissolved in approximately 30 ml. of liquid ammonia. Enough sodium is added, in small pieces, to give a permanent blue color, indicating a slight excess of sodium. To this solution is added, with stirring, 0.6 g. of methyl iodide. The liquid

ammonia is then allowed to evaporate spontaneously. The white residue is dissolved in 10 ml. of water and acidified to Congo red with 30% hydrobromic acid, and the solution is filtered. To the filtrate are added 1 ml. of pyridine and 3 volumes of boiling ethanol, and the mixture is cooled in an ice-bath. The precipitate is collected and washed with a mixture of alcohol and water (3:1). The crude product, which melts at 268–271°, is dissolved in 6 ml. of water with heating, and 3 volumes of hot alcohol are added.

As the solution cools, glistening plate-like crystals of H₃²-methionine appear. The yield of recrystallized product is 0.214 g., m.p. 275–276° (cor.). Further recrystallization does not raise the melting point, and a mixed melting point of the product with an authentic sample of methionine, m.p. 275–276° (cor.), is 275–276° (cor.).

C. Other Preparations

C¹⁴₁-H₃²-Methionine, [2-amino-4-(methylthio-C¹⁴₁-H₃²)butyric acid] has been prepared,³ according to a procedure⁴ similar to that described, from methyl-C¹⁴₁-H₃² iodide and S-benzylhomocysteine.

¹V. du Vigneaud, H. M. Dyer and J. Harmon, *J. Biol. Chem.*, **101**, 719 (1933).

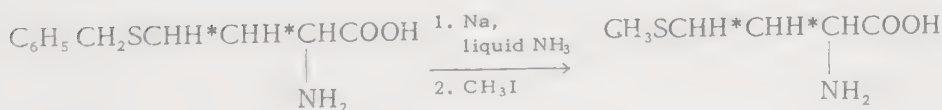
²L. W. Butz and V. du Vigneaud, *ibid.*, **99**, 135 (1932).

³J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, *ibid.*, **215**, 593 (1955).

⁴D. B. Melville, J. R. Rachele and E. B. Keller, *ibid.*, **169**, 419 (1947).

METHIONINE-3,4-H₂²

[2-Amino-4-(methylthio)butyric-3,4-H₂² Acid]



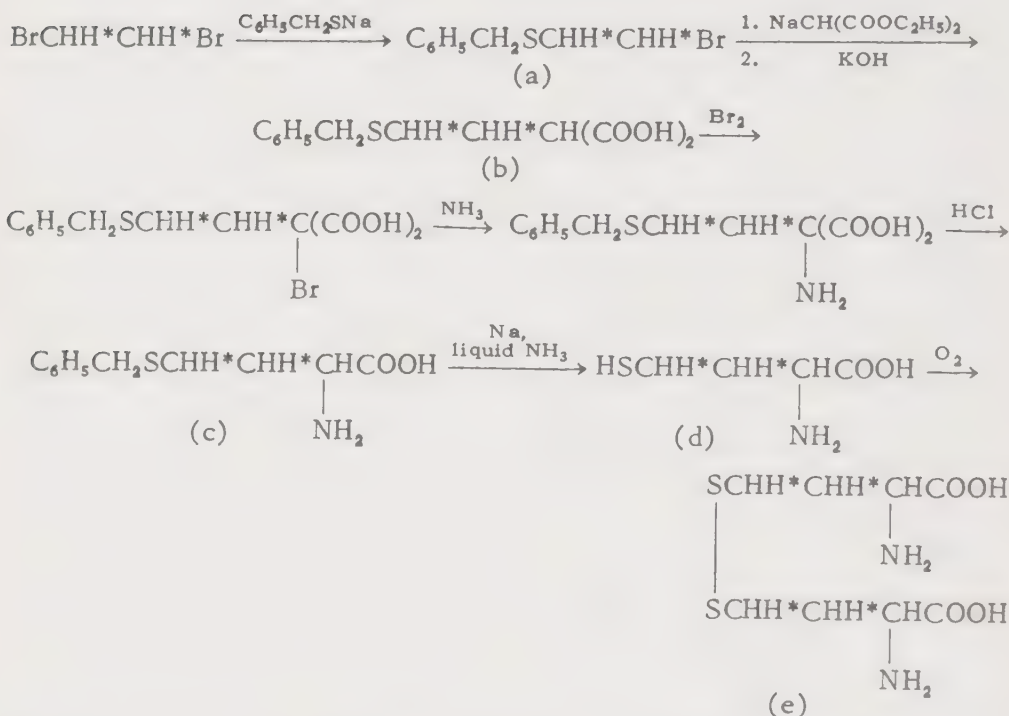
W. I. Patterson and V. du Vigneaud, *J. Biol. Chem.*, **123**, 327 (1938).

Procedure

To 60 ml. of liquid ammonia in a 3-necked flask, equipped with a mercury-sealed stirrer and cooled in a mixture of trichloroethylene and Dry Ice, is added 8.5 g. of 2-amino-4-(benzylthio)butyric-3,4-H₂² acid. Metallic sodium is added in small portions until a blue color persists. The blue color is discharged with 0.3 ml. of methyl iodide which is followed by a further quantity of 2.4 ml. (1.05 moles). After evaporation of the liquid ammonia, the residue is dissolved in 15 ml. of water and

made just alkaline to litmus with 45% hydriodic acid. A halogen-free product, 4.5 g., precipitates. After concentration of the filtrate almost to dryness, addition of 200 ml. of absolute alcohol, and cooling of the solution overnight, an additional 0.9 g. of product is obtained. Recrystallization of the two fractions from water and alcohol affords 4.4 g. (78%) of halogen-free methionine-3,4- H_2^2 .

4,4'-DITHIOBIS(2-AMINOBUTYRIC-3,4- H_2^2 ACID)
(H_2^2 -Homocystine)



W. I. Patterson and V. du Vigneaud, *J. Biol. Chem.*, **123**, 327 (1938); *ibid.*, **111**, 393 (1935).

A. Procedure

(a) *Benzyl 2-Bromoethyl-1,2- H_2^2 Sulfide*. Metallic sodium, 5 g., is added to 40 ml. of methyl alcohol in a 300-ml. flask equipped with a reflux condenser and drying tube. As soon as the vigorous reaction has somewhat subsided, 25 g. of α -toluenethiol is added. When the sodium has all dissolved, the solution is cooled in an ice-bath and added to 199 g. of ethylene-1,2- H_2^2 bromide, cooled to its freezing point in a 1-l. flask. The two solutions are mixed and kept in an ice-bath, and quite soon a vigorous reaction begins (Note 1). When the reaction subsides,

300 ml. of water is added, and the mixture is distilled at 70 mm. until all the unchanged ethylene-1,2- H_2^2 bromide is recovered. The above condensation is then repeated with the recovered material, 152 g., using 5 g. of sodium and 25 g. of α -toluenethiol. The unchanged ethylene-1,2- H_2^2 bromide recovered, 113 g., is used in a third condensation with 4.1 g. of sodium and 22 g. of α -toluenethiol (Note 2). The residues from the three vacuum distillations are combined and distilled at 1 mm. pressure. The fraction boiling from 115–130°, consisting chiefly of benzyl 2-bromoethyl-1,2- H_2^2 sulfide, is redistilled. With an oil-bath temperature of 150°, the fraction boiling at 118–123° (1 mm.) weighs 55 g. (41%).

(b) [2-(Benzylthio)ethyl-1,2- H_2^2]malonic Acid. To a solution of 5.5 g. of sodium in 100 ml. of absolute alcohol is added 60 g. of malonic ester. The solution is cooled until precipitation begins, and then 55 g. of benzyl 2-bromoethyl-1,2- H_2^2 sulfide is added. After the mixture stands at room temperature for 1 hour, it is refluxed for 3 hours. The ester is then hydrolyzed by refluxing the mixture with 70 g. of potassium hydroxide in 300 ml. of 50% alcohol. The alcohol is removed *in vacuo*, and the residual solution, cooled below 20°, is acidified with concentrated hydrochloric acid, in the presence of 200 ml. of alcohol-free ether. After the ether layer is separated, and the aqueous layer is washed with ether, to the combined ether solution is added 200 ml. of benzene. Ether is removed under reduced pressure until crystallization begins. After the mixture has cooled in an ice-bath for a short time, the product is collected on a filter, washed with benzene and air-dried. The product, m.p. 119–120°, weighs 28.5 g.; concentration of the mother liquor affords 11 g. more of the acid melting a few degrees lower. The total yield, 39.5 g., is 65% of the theoretical amount.

(c) 2-Amino-4-(benzylthio)butyric-3,4- H_2^2 Acid, (H_2^2 -S-Benzylhomocysteine). [2-(Benzylthio)ethyl-1,2- H_2^2]malonic acid, 28.5 g., is dissolved in 225 ml. of dry ether, and 1 ml. of dry bromine is added to the solution cooled in an ice-bath. As soon as this bromine has reacted, 4.6 ml. of bromine is added during 15 minutes. The reaction mixture is then immediately poured into 290 ml. of cold concentrated ammonium hydroxide, with efficient stirring. A heavy precipitate separates which, with occasional stirring, goes into solution in about 24 hours. After an additional 24 hours at room temperature, excess ammonia is removed from the solution of amino-[2-(benzylthio)ethyl-1,2- H_2^2]malonic acid *in vacuo*. Then, 45 ml. of concentrated hydrochloric acid is added, and the mixture is heated in a boiling water-bath for 1 hour. After the mixture cools slightly, it is neutralized with concentrated ammonium hydroxide until acid to litmus, but alkaline to Congo red indicator. The mixture is cooled in ice and filtered, and the solid product is washed with water

and then 95% alcohol until the filtrate does not yield a turbidity with water. The dry product weighs 16.8 g. (66%) (Note 3).

(d) *2-Amino-4-mercaptobutyric-3,4-H₂² Acid, (H₂²-Homocysteine)*. To 60 ml. of liquid ammonia in a 3-necked flask, equipped with a mercury-sealed stirrer and cooled in a mixture of trichloroethylene and Dry Ice, is added 8.25 g. of 2-amino-4-(benzylthio)butyric-3,4-H₂² acid. Sodium is introduced into the flask in small portions until a blue color persists at the boiling point of liquid ammonia. Then, 3.5 g. of ammonium chloride is added, and the ammonia is evaporated. The residue is treated with 40 ml. of water, and the solution is taken directly to the next step (Note 4).

(e) *4,4'-Dithiobis(2-aminobutyric-3,4-H₂² Acid), (H₄²-Homocysteine)*. The solution of H₂²-homocysteine, to which is added a little ferric chloride as catalyst, is oxidized with a stream of air. When the test for the sulfhydryl group with nitroprusside reagent becomes negative, the mixture is made neutral to litmus, causing the product to precipitate. The crude product is dissolved in the minimum amount of dilute sodium hydroxide, filtered and precipitated by neutralization of the solution with hydrochloric acid. The yield is 4.4 g. (90%).

B. Notes

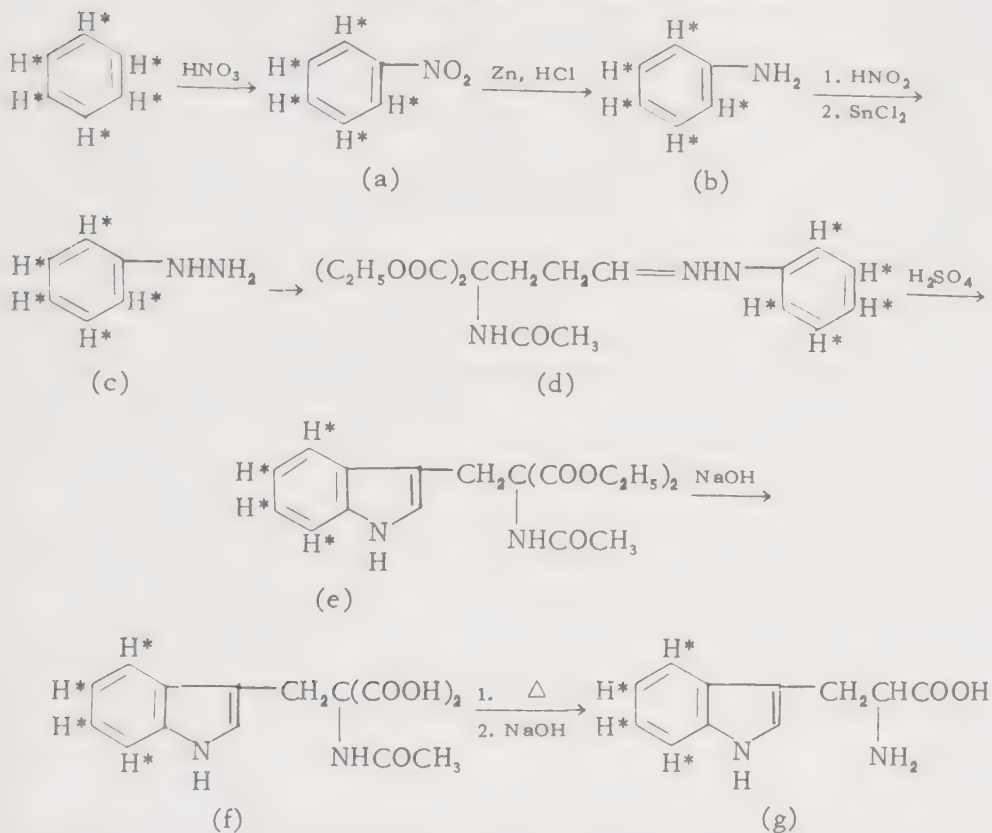
1. To obtain the best yield, the reaction is allowed to proceed vigorously, but the rate of the reaction is controlled by cooling to prevent the solution from boiling over. It should be mentioned that benzyl 2-haloethyl sulfides are strong skin vesicants.

2. The unchanged ethylene-1,2-H₂² bromide from the third condensation amounted to 78 g. It should be noted that, according to the earlier reference, Patterson and du Vigneaud obtained a 71-75% yield of benzyl 2-chloroethyl sulfide in a single condensation, using ethylene chloride.

3. The product was indistinguishable from ordinary *S*-benzylhomocysteine.

4. A small amount of unchanged *S*-benzylhomocysteine is removed from the solution.

TRYPTOPHAN-4,5,6,7- H_4^2
(α -Amino-3-indolepropionic-4,5,6,7- H_4^2 Acid)



R. W. Schayer and L. M. Henderson, J. Biol. Chem., 195, 657 (1952).

A. Procedure

(a) *Nitrobenzene- H_5^2* . To a shaken mixture of 58 ml. of concentrated nitric acid and 58 ml. of fuming nitric acid (sp. gr. 1.50), maintained between 40–50°, is added 41 g. of benzene- H_6^2 in small portions, during 30 minutes (Note 1). The lower layer of acid is removed in a separatory funnel, and the nitrobenzene- H_5^2 is washed with water, dilute sodium hydroxide and again with water. The product is dried over calcium chloride and distilled; yield, 47 g.

(b) *Aniline-2,3,4,5,6- H_5^2* . The 47 g. of nitrobenzene- H_5^2 is reduced to aniline-2,3,4,5,6- H_5^2 according to the procedure described by Fieser,¹ with granulated tin and hydrochloric acid. Following the reduction, the solution is made alkaline with sodium hydroxide, and the product is isolated by steam distillation. After the distillate is saturated with sodium chloride, the aniline is extracted into ether and dried over solid sodium hydroxide. Following removal of the ether, distillation of the product yields 32.4 g. of aniline-2,3,4,5,6- H_5^2 .

(c) *Phenyl-H₅²-hydrazine*. By adaptation of the procedure described by Hickinbottom,² a solution of 32.4 g. of aniline-2,3,4,5,6-H₅² in 650 ml. of concentrated hydrochloric acid, maintained at 0-5°, is diazotized by the addition of 26 g. of sodium nitrite dissolved in 160 ml. of water. An ice-cold solution of 162 g. of stannous chloride in 145 ml. of concentrated hydrochloric acid is added to the cold solution of diazonium salt. The precipitate of phenyl-H₅²-hydrazine hydrochloride is collected after about 0.5 hour. The free base is liberated with an excess of sodium hydroxide solution and dissolved in ether. After the solution is dried, ether is removed, and the product is distilled under reduced pressure (Note 2); the yield of phenyl-H₅²-hydrazine is 25.9 g.

(d) *1-(4-Acetamido-4,4-diethoxycarbonylbutylidene)-2-phenyl-H₅²-hydrazine*, [Ethyl Acetamido(3-phenyl-H₅²-hydrazonopropyl)malonate]. According to the following procedure of Moe and Warner,³ 4-acetamido-4,4-dicarbethoxybutyraldehyde, prepared from 217 g. of ethyl acetamidomalonate and 68.5 ml. of acrolein, in 400 ml. of benzene, is treated with 24 ml. of glacial acetic acid and 120 g. of phenylhydrazine. After being warmed to 50°, the resulting orange-colored solution is set aside for 2 days. The crystalline product is collected by filtration and washed with 150 ml. of benzene. The product is further decolorized by suspension in 250 ml. of benzene; then is collected and dried, *in vacuo*. The yield of phenylhydrazone derivative is 315.1 g. (87%); m.p. 140-141° (Note 3).

(e) *Ethyl α-Acetamido-α-carbethoxy-3-indolepropionate-4,5,6,7-H₄²*. Cyclization of the above phenylhydrazone derivative is done according to the following procedure of Warner and Moe.⁴ To 300 ml. of water, containing 14 ml. of concentrated sulfuric acid, is added 50 g. of ethyl acetamido(3-phenylhydrazonopropyl)malonate (Note 4). With very vigorous stirring, the reaction mixture is heated to reflux. The phenylhydrazone liquifies at this temperature and, after approximately 1 hour, the suspended liquid solidifies. The reflux temperature is maintained for a period of 4.5 hours. After cooling, the solid reaction product is mixed with water in a Waring Blendor; then is collected, washed with water and dried *in vacuo*. The yield of product, m.p. 145-149°, is 42.5 g. (about 90%). After recrystallization from aqueous ethanol (1:1), the yield of product, m.p. 156-157°, is 35 g. (73%).

(f) *α-Acetamido-α-carboxy-3-indolepropionic-4,5,6,7-H₄² Acid*. According to the procedure of Snyder and Smith,⁵ 33.62 g. (0.097 mole) of the above ester is heated under reflux for 4 hours with 19.2 g. (0.48 mole) of sodium hydroxide in 192 ml. of water. After treatment with charcoal, the solution is cooled in an ice-bath, and 50 ml. (0.6 mole) of cold concentrated hydrochloric acid is added with cooling so that the temperature of the solution does not exceed 25°. The solution is cooled for 4 hours,

and the light pink precipitate is collected and dried over calcium chloride in an evacuated desiccator for 14 hours. The yield of crude product, m.p. 136–139°, is 32 g. (Note 5).

(g) *Tryptophan-4,5,6,7-H₄²*, (α -Amino-3-indolepropionic-4,5,6,7-H₄² Acid). A mixture of 28 g. of crude α -acetamido- α -carboxy-3-indolepropionic-4,5,6,7-H₄² acid and 120 ml. of water is refluxed for 2.5 hours⁵ (Note 6). After the decarboxylation is complete, 16 g. (0.4 mole) of sodium hydroxide in 40 ml. of water is added, and the solution is refluxed for 20 hours. The alkaline solution is treated with carbon and acidified with 24 g. (0.4 mole) of acetic acid. A white precipitate forms immediately; after the solution is refrigerated for 12 hours, the solid product is collected. The product is dissolved in 200 ml. of water containing 5 g. of sodium hydroxide and is treated with carbon. Then, 100 ml. of 95% ethanol is added to the solution, which is warmed to 70°, acidified with 7.5 ml. of acetic acid and cooled slowly. The product, which crystallizes in flat plates, is collected and washed successively with two 40-ml. portions of water, two 40-ml. portions of alcohol and two 30-ml. portions of ether. The yield of product, m.p. 272–280° (dec.), is 14 g. (81% based on compound (e), above) (Note 7).

B. Notes

1. Sulfuric acid was not used in the nitration mixture to avoid exchange of hydrogen for deuterium.

2. The boiling point of phenylhydrazine is 120° (12 mm.).⁶

3. The crystals were nearly colorless.

4. Cyclization was also effected with sulfuric acid in absolute ethanol and with boron trifluoride in glacial acetic acid. The yields were lower than in the procedure described.

5. The acid can be purified by dissolution in 10 volumes of water (containing a trace of sodium hydrosulfite) at 50° and cooling the solution. After two recrystallizations from 20% ethanol, a sample melted at 144.5° (dec.).

6. The *N*-acetyltryptophan produced is less soluble than the starting acid and may partially crystallize from the solution as the reaction proceeds.

7. An analytical sample was twice recrystallized from 33% ethanol, m.p. 275–282° (dec.)

C. Other Preparations

Aniline-2,3,4,5,6-H₅² has been prepared⁷ by exchange upon heating a mixture of aniline, water-H₂², sodium hydroxide-H² and Raney nickel-aluminum alloy. The following derivatives were also prepared by ex-

change: acetanilide-2',3',4',5',6'-H₅², 2,4,6-tribromoaniline-3,5-H₂² and 4'-bromoacetanilide-2',3',5',6'-H₄². Analytical data indicated that the distributions of deuterium in the *ortho*-, *meta*- and *para*- positions of the exchanged aniline were, respectively, about 75%, 11.5% and 13.4%.

¹L. F. Fieser, *Experiments in Organic Chemistry*, 2nd ed., Heath and Co., New York, 1941, p. 149.

²W. J. Hickinbottom, *Reactions of Organic Compounds*, Longmans, Green and Co., New York, 1936, p. 355.

³O. A. Moe and D. T. Warner, *J. Am. Chem. Soc.*, 70, 2763 (1948).

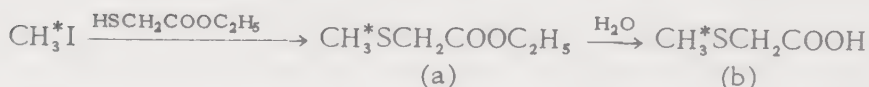
⁴D. T. Warner and O. A. Moe, *ibid.*, 70, 2765 (1948).

⁵H. R. Snyder and C. W. Smith, *ibid.*, 66, 350 (1944).

⁶V. Meyer and M. T. Lecco, *Ber.*, 16, 2976 (1883).

⁷W. M. Lauer and L. A. Errede, *J. Am. Chem. Soc.*, 76, 5162 (1954).

(METHYLTHIO-H₃²)ACETIC ACID



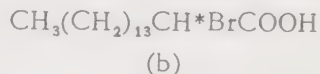
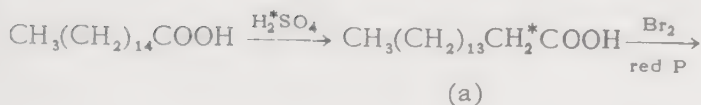
G. A. Maw and V. du Vigneaud, *J. Biol. Chem.*, 176, 1037 (1948).

Procedure

(a) *Ethyl (Methylthio-H₃²)acetate*. To a solution of 0.46 g. of sodium in 7 ml. of absolute methanol at -10° is added 2.4 g. of ethyl mercaptoacetate. Then 3.0 g. of methyl-H₃² iodide is added gradually. The resulting solution gives a negative test for the sulfhydryl group. The methanol is evaporated, leaving a mixture of sodium iodide and ethyl (methylthio-H₃²)acetate.

(b) *(Methylthio-H₃²)acetic Acid*. The ethyl (methylthio-H₃²)acetate is heated with 3 ml. of water which is kept alkaline to Bromothymol blue by the dropwise addition of 15% potassium hydroxide solution. Following the hydrolysis, the solution is acidified with concentrated hydrochloric acid, and the product is extracted with two 7-ml. portions of ether. After the ether solution is dried over sodium sulfate, the ether is removed, and the (methylthio-H₃²)acetic acid is distilled; b.p. 112-113° (12 mm.).

2-BROMOHEXADECANOIC-2-H² ACID
(2-Bromopalmitic-2-H² Acid)



W. E. von Heyningen, D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, **125**, 495 (1938).

A. Procedure

(a) *Hexadecanoic-2-H₂² Acid*, (*Palmitic-2-H₂² Acid*) (Note 1). Palmitic acid, 1.0 g., and 1.85 g. of 100% sulfuric acid-H₂² (Note 2) are weighed into a test tube, and 0.186 ml. of 95% water-H₂² is added to bring the sulfuric acid concentration to 90%. After the neck of the tube is drawn to a long capillary and sealed, the tube is placed in an oven at 98–100° for 50 hours. When the tube is opened, the dark amber contents are diluted with water, and the organic acid is extracted into ether. The ethereal solution is extracted with alcoholic potassium hydroxide, the alkaline solution is acidified with dilute sulfuric acid and extracted again with ether (Note 3). After the extract is well dried, ether is removed, and the acid is recrystallized from aqueous acetone. The yield of hexadecanoic-2-H₂² acid is 913 mg., m.p. 62.5° (Note 4).

(b) *2-Bromohexadecanoic-2-H² Acid*, (*2-Bromopalmitic-2-H² Acid*). Hexadecanoic-2-H₂² acid, 1.5 g., is ground with 0.12 g. of red phosphorus and transferred to a flask fitted with a capillary dropping funnel and attached to a condenser by means of a ground glass joint. During 20 minutes, the mixture is treated dropwise with 1.5 ml. of bromine and then heated on a steam-bath for 1.5 hours. The reaction mixture is poured into dilute sodium sulfite solution. The precipitate is dissolved in ether, and this solution is washed with water and dried. After removal of ether, the acid is recrystallized from light petroleum; the product, 1.34 g., melts at 53–54° (Note 5).

B. Notes

1. The preparation of fatty acids containing deuterium by treatment of the acids with hot concentrated sulfuric acid-H₂² is a general method (also see H²-lauric acid). Saturated fatty acids are very resistant to hot sulfuric acid, and the loss of organic matter after such treatment is very small. The deuterium thus introduced is stably bound; it is not removed from the fatty acids by treatment with boiling 20% sulfuric acid or boiling 7% aqueous alcoholic potassium hydroxide.

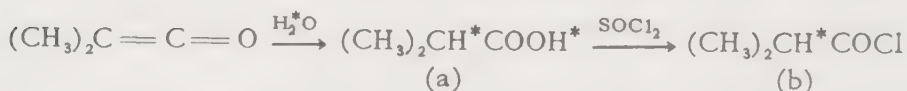
2. Concentrated sulfuric acid- H_2^2 , 100% with respect to acid and containing 95 atom per cent deuterium, is prepared by dissolving one mole of sulfur trioxide in one mole of 95% water- H_2^2 .

3. By this means the labile deuterium of the carboxyl group is replaced by hydrogen.

4. The deuterium content was 4.32 atom per cent; this corresponds to an exchange of 1.38 atoms per molecule. In similar experiments using 1-g. quantities of palmitic acid with 95 and 98% sulfuric acid- H_2^2 at 98-100° and 90% sulfuric acid- H_2^2 at 130°, the yields of product and atom per cent deuterium were respectively: 764 mg. and 5.04%, 593 mg. and 4.62%, and 933 mg. and 4.6%.

5. The deuterium content of the product was 2.48 atom per cent. Assuming that all the deuterium in the original acid, 4.65 atom per cent, was at C-2, half should have been removed by the bromination, and the calculated value was 2.40 atom per cent.

2-METHYLPROPIONYL-2- H^2 CHLORIDE



C. C. Price and H. Morita, J. Am. Chem. Soc., 75, 3686 (1953).

A. Procedure

(a) *2-Methylpropionic-2- H^2 Acid- H^2* . Dimethylketene is prepared essentially by the procedure of Staudinger and Klever¹ as modified by vanAlphen² (Note 1). A 1.5-l. three-necked flask is fitted with a 1-l. dropping funnel, a water-cooled Allihn condenser attached to a receiver immersed in ice, and a nitrogen inlet. Clean 20-mesh zinc (65 g., 1.0 g. atom) is placed in the flask, and the apparatus is swept with dry nitrogen. A solution of 115 g. (0.5 mole) of 2-bromo-2-methylpropionyl bromide in 550 ml. of dry ether is added dropwise onto the zinc (Note 2). The reaction begins with vigorous boiling of the ether, and the distillate consists of a yellowish-green solution of dimethylketene in ether. By occasional mild heating the reaction is kept in progress. When the reaction is completed, the colored solution is redistilled, into a cooled receiver, in order to remove traces of hydrogen bromide and isobutyryl bromide.

The distillate is then treated with 4 ml. of water- H_2^2 (90% deuterium), dried over anhydrous sodium sulfate and fractionally distilled through a Vigreux column in a stream of dry nitrogen. The yield of 2-methylpropionic-2- H^2 acid- H^2 (b.p. 151-153°) is 8.9 g. (20%), n_D^{25} 1.3884.

(b) *2-Methylpropionyl-2-H² Chloride*. 2-Methylpropionic-2-H² acid-H², 100 g. (b.p. 150° at 743.5 mm.), is reacted with 165 g. of freshly distilled thionyl chloride.³ The yield of the acid chloride, b.p. 90–91°, is 84.1 g. The product is fractionally distilled twice through a 40-cm. glass-helix packed column provided with a partial take-off head (Note 3).

B. Notes

1. This procedure proved more satisfactory than the preparation and pyrolysis of dimethylketene dimer⁴ or the pyrolysis of *N*-isobutyryl-phthalimide.⁵

2. In many instances, the reaction was initiated more readily by adding a drop of water to the zinc prior to addition of the ether solution.

3. Density of a middle fraction, b.p. 90.3° (743.5 mm.), was 1.0139 ± 0.0004; n_D^{25} 1.4042.

¹H. Staudinger and H. W. Klever, *Ber.*, 39, 968 (1906).

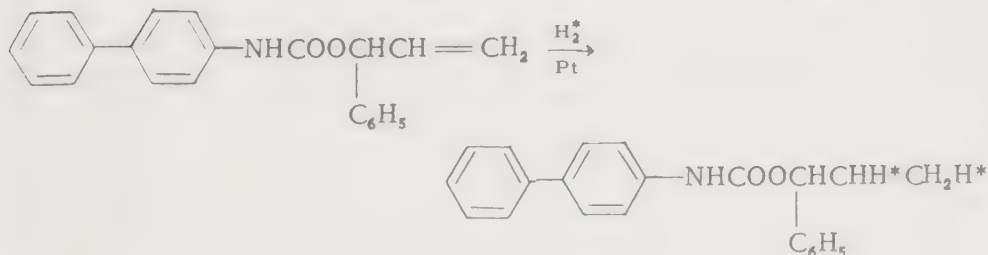
²J. vanAlphen, *Rec. trav. chim.*, 43, 823 (1925).

³*Organic Syntheses*, Vol. 25, Wiley, New York, 1945, p. 58.

⁴L. L. Miller and J. R. Johnson, *J. Org. Chem.*, 1, 135 (1936).

⁵C. D. Hurd and M. F. Dull, *J. Am. Chem. Soc.*, 54, 2432 (1932).

(+)- α -ETHYL-1,2-H²-BENZYL 4-PHENYLCARBANILATE



J. B. M. Coppock, J. Kenyon and S. M. Partridge, *J. Chem. Soc.*, 1938, 1069.

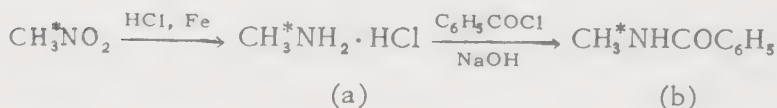
Procedure

(+)- α -Vinylbenzyl 4-phenylcarbanilate, 10 g., in 250 ml. of ether is reduced with hydrogen-H² (98%) at 2 atmospheres in the presence of platinum oxide catalyst.¹ After an induction period of 10 minutes, the reaction proceeds rapidly and is complete in about 15 minutes. The catalyst is removed, light petroleum is added to the filtrate, and the product separates in long needles, m.p. 137.3–137.5°. After four recrystallizations from benzene-light petroleum, the product, 2.8 g., melts at 137.9–138.4°. Recrystallization of second crops gives 2 g. of product melting at 138.0–138.3°, and 2 g. more, m.p. 136.5–137.5°, is obtained

from the mother liquors. There is no significant difference between the rotatory power of these fractions.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

N-METHYL-H₃²-BENZAMIDE



H. D. Noether, *J. Chem. Phys.*, 10, 664 (1942).

A. Procedure

(a) *Methylamine-1-H₃² Hydrochloride*. Nitromethane-H₃² is reduced according to the procedure of Krause.¹ Nitromethane-H₃² and hydrochloric acid, in the molar ratio of 1 to 1.5, are heated together with iron filings at 70° for 1 hour, under a hydrogen atmosphere (Note 1). The solution is then made strongly alkaline, and the free methylamine-1-H₃² is distilled into an excess of hydrochloric acid. The solution of methylamine-1-H₃² hydrochloride is evaporated to dryness.

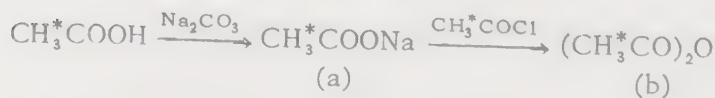
(b) *N-Methyl-H₃²-benzamide*. To a mixture of 20 ml. of 5% sodium hydroxide solution, 5 ml. of chloroform and 0.5 ml. of benzoyl chloride is added 0.5 g. of methylamine-1-H₃² hydrochloride, with stirring. The mixture is stirred for about 30 minutes and then set aside for several hours. The chloroform layer is separated, and the aqueous layer is extracted with chloroform. The combined chloroform solutions are washed with water, dried with anhydrous magnesium sulfate and evaporated to a small volume. Hexane is stirred into the solution, and the product is collected and washed with hexane. The product is then dissolved in ether and reprecipitated by the addition of ligroin.

B. Notes

1. If air is excluded and the medium is kept acidic, to completion of the reaction, the reduction is nearly quantitative. Carbon dioxide and oxygen are also removed from the water used in the reaction.

¹H. Krause, *Chem. Ztg.*, 40, 810 (1916); *Chem. Abstracts*, 11, 1650 (1917).

BIS(ACETIC-H₃²) ANHYDRIDE



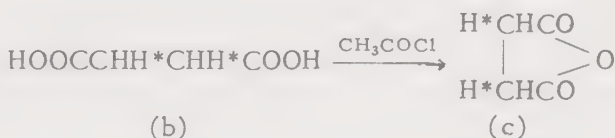
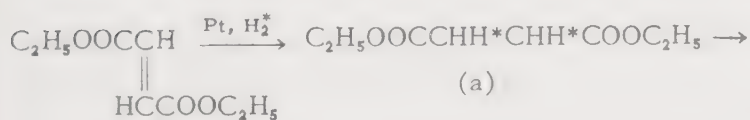
B. Nolin and R. N. Jones, *Can. J. Chem.*, 30, 727 (1952).

Procedure

(a) *Sodium Acetate-H₃²*. Acetic-H₃² acid is neutralized with sodium carbonate solution. The solution is evaporated to dryness, and the resultant sodium acetate-H₃² trihydrate is decomposed at 160° under vacuum.

(b) *Bis(acetic-H₃²) Anhydride*. Then, 1 equivalent of acetyl-H₃² chloride is added dropwise to the dry sodium acetate-H₃², and the anhydride is distilled; b.p. 137.0–138.2°, n_D^{20} 1.3881.

SUCCINIC-2,3-H₂² ANHYDRIDE



M. T. Leffler and R. Adams, J. Am. Chem. Soc., 58, 1552 (1936).

A. Procedure

(a) *Ethyl Succinate-2,3-H₂²*. The reduction with hydrogen-H₂² is carried out in the usual shaking type of apparatus¹ with modifications to accommodate small amounts of materials.²

Each reduction tube is charged with 4.00 g. of ethyl fumarate, 0.05 g. of platinum catalyst³ and 7 ml. of dry ethyl acetate (Note 1). An excess of nearly 100% hydrogen-H₂² is available to the reaction mixture. The reduction is complete after 8 hours, and no more hydrogen-H₂² is absorbed in 3 additional hours. The catalyst is collected on a filter and washed with solvent. The ethyl acetate is then removed from the combined filtrates at 60° under reduced pressure. The residue is distilled *in vacuo*; b.p. 126–126.5° (40 mm.) and 106–106.5° (16 mm.), n_D^{20} 1.4193, d_4^{20} 1.0533 (Note 2). The yield of ethyl succinate-2,3-H₂² is 3.4–3.7 g. (83–91%) (Note 3).

(b) *Succinic-2,3-H₂² Acid*. The ethyl succinate-2,3-H₂² is hydrolyzed by heating 10 g. of the ester under reflux with 100 ml. of water containing 3 drops of concentrated nitric acid. The clear solution is cooled, and the nitric acid is neutralized with the calculated amount of sodium carbonate. Evaporation of the solution to dryness, *in vacuo* at 40–50°, gives 6.6 g. of crude acid, which is recrystallized from water, m.p. 184–184.5° (Note 4).

(c) *Succinic-2,3- H_2^2 Anhydride*. Succinic-2,3- H_2^2 acid, 2.36 g., is heated under reflux for 3 hours with 4.7 g. of acetyl chloride. With slow cooling of the clear solution, colorless prisms of the anhydride are formed. After cooling in ice-water, the product is collected, washed with cold, absolute ether and dried over soda-lime *in vacuo*, m.p. 119.3–119.6°.

B. Notes

1. Acetic acid-free.
2. The boiling point was constant and gave no indication of the presence of isomers.
3. Ethyl succinate-2,3- H_2^2 was also prepared from ethyl maleate in 88% yield. There was no apparent difference in the two products.
4. The product was fractionally crystallized from hot water, and each fraction was recrystallized. No differences were noted. The products from fumarate and maleate were identical. All the experimental evidence indicates symmetry of the carbon $RR'CHH^2$. In order to compare the optical activity of several deuterium and hydrogen analogs, the following alkaloid salts of succinic-2,3- H_2^2 acid were prepared: brucine, m.p. 216–218°(dec.); quinine, m.p. 198–201° (after drying 24 hours *in vacuo* at 100°); strychnine, m.p. 210° (after drying at 100° *in vacuo*).

C. Other Preparations

Erlenmeyer⁴ prepared succinic-2,3- H_2^2 acid in 90% yield by the simultaneous hydrolysis and decarboxylation of ethyl 1,1,2,2-ethanetetra-carboxylate at 150° with water- H_2^2 (33%). Succinic-2,3- H_2^2 acid has also been prepared: by hydrogenation of ethyl fumarate and hydrolysis of the resulting ethyl succinate-2,3- H_2^2 ,^{4,5} by hydrogenation of maleic acid⁶ and from glutamic-2,3- H_2^2 acid resulting from the hydrogenation of 2-oxo-glutaric acid.⁶

Succinic- H_4^2 anhydride has been prepared² by heating a mixture of succinic- H_4^2 acid and phosphorus oxychloride.

¹*Organic Syntheses*, Coll. Vol. I, 2nd. ed., Wiley, New York, 1941, p. 61.

²A. McLean and R. Adams, *J. Am. Chem. Soc.*, 58, 804 (1936).

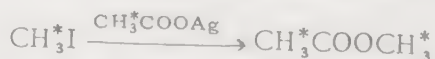
³*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

⁴H. Erlenmeyer, W. Schoenauer and H. Sullmann, *Helv. Chim. Acta*, 19, 1376 (1936).

⁵E. O. Weinmann, M. G. Morehouse and R. J. Winzler, *J. Biol. Chem.*, 168, 717 (1947).

⁶D. Rittenberg, S. Ratner and H. D. Hoberman, *J. Am. Chem. Soc.*, 62, 2249 (1940).

METHYL- H_3^2 ACETATE- H_3^2



B. Nolin, *Can. J. Chem.*, 32, 1 (1954).

A. Procedure

By the use of a vacuum apparatus, 35–55 mmoles of iodomethane- H_3^2 is transferred into a flask which contains silver acetate- H_3^2 in 10% excess. The flask is removed from the manifold and attached to a reflux condenser. The mixture is heated overnight at 65–75°. The volatile material is distilled *in vacuo* and is again treated with fresh silver acetate- H_3^2 (Note 1). This process is repeated a third time. The resulting ester is treated twice with small amounts of phosphorus pentoxide (Note 2). A small fraction is distilled *in vacuo* from a Dry Ice-acetone bath; then a large middle fraction is collected in the same manner. The yield of pure product averages 50%, and its vapor pressure is practically constant at 0°.

Methyl- H_3^2 acetate and methyl acetate- H_3^2 are prepared similarly from the appropriate reagents. Physical properties of these compounds are shown in Table XVI, 3.

B. Notes

1. In the second treatment the number of mmoles of silver acetate- H_3^2 was 0.3 of the original amount of iodomethane- H_3^2 . Usually no yellow color developed during the second treatment, and the reaction was considered complete; but in this instance a third treatment appeared desirable.

2. Some loss is incurred in this treatment.

C. Other Preparations

Methyl- H_3^2 acetate- H_3^2 has been prepared^{1,2} by the esterification of methanol- H_4^2 with acetic- H_3^2 acid- H^2 in the presence of sulfuryl chloride for catalyst. The use of sulfuryl chloride as catalyst, instead of the usual hydrochloric or sulfuric acids, eliminates isotopic dilution. The product,² separated by fractional distillation and refluxed with silver oxide to remove traces of chlorine, distilled between 55.5 and 56.5°; n_D^{15} 1.3605, n_D^{20} 1.3578.

¹M. Corval and J. Lecomte, *Mikrochim. Acta*, 1955, 25.

²M. Corval and C. Piolet, *Bull. soc. chim. France*, 21, 234 (1954).

TABLE XVI, 3

Deuterated Methyl Acetates

Compound	Formula	H ² atoms per molecule		Vapor pressure, mm., 0°C.	n_D^{20}
		Found	Required		
Methyl acetate	$\text{CH}_3\text{COOCH}_3$	—	—	62.4	1.3616
Methyl- H_3^2 acetate	$\text{CH}_3\text{COOCH}_3^2$	2.97	3	63.2	1.3607
Methyl acetate- H_3^2	$\text{CH}_3^2\text{COOCH}_3$	2.97	3	63.4	1.3603
Methyl- H_3^2 acetate- H_3^2	$\text{CH}_3^2\text{COOCH}_3^2$	5.95	6	64.0	1.3595

ETHYL- H_5^2 ACETATE- H_3^2 

B. Nolin, Can. J. Chem., 31, 1257 (1953).

A. Procedure

(a) *Iodoethane- H_5^2* . Deuterated ethyl iodides are prepared from the corresponding bromides by a modification of the method of Spindler¹ (Note 1). Dry bromoethane- H_5^2 and dry calcium iodide, in excess, are placed in a tube which is evacuated and sealed. The tube is heated for 120 hours in a bath at 70–75°. After the mixture of ethyl bromide and iodide is treated, in the same manner, with a second portion of calcium iodide, the conversion to ethyl iodide is practically complete. 1-Iodoethane-1- H_2^2 and 1-iodoethane-2- H_3^2 are prepared in the same manner from the corresponding bromides (Note 2).

(b) *Ethyl- H_5^2 Acetate- H_3^2* (Note 3). Ethyl- H_5^2 iodide is transferred, in a vacuum system, to a small flask containing silver acetate- H_3^2 . The flask is removed from the manifold, attached to a reflux condenser and heated overnight at 95°. The volatile material is distilled *in vacuo* from the inorganic residue (Note 4). The ester, now free of ethyl- H_5^2 iodide, is treated three times with small amounts of phosphorus pentoxide, or until the latter is not discolored within 5 minutes. The product is frozen and degassed, a small light fraction is removed, and the remainder, which has a nearly constant vapor pressure at 0°, is vacuum-distilled. The yield is 2.0 g. (75%). The refractive index, vapor pressure at 20° and isotopic purity, expressed in deuterium atoms per molecule, are listed in the following table, together with the data on six partially deuterated ethyl acetates (Note 5), prepared by the above procedure.

B. Notes

1. The preparation of ethyl- H_5^2 iodide is given as an example of this general method of preparing alkyl iodides.
2. As shown by mass spectrometric analyses, the isotopic purity of each of these iodides was at least 99.2%.
3. This is a general method of preparing deuterated esters.
4. If the product is found by mass spectrometric analysis to contain unreacted ethyl- H_5^2 iodide, the mixture is treated as before with some fresh silver acetate- H_3^2 .
5. The vapor pressures were measured with an apparatus similar to that described by Booth and Swinehart;² they are higher than the vapor pressure of normal ethyl acetate.

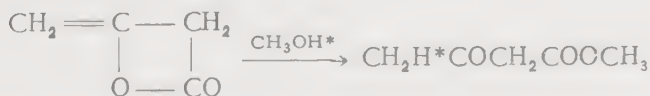
¹H. Spindler, Ann., 231, 257 (1885).

²H. S. Booth and C. F. Swinehart, J. Am. Chem. Soc., 57, 1333 (1935).

TABLE XVI, 4

Deuterated Ethyl Acetates

Compound	Formula	H ² atoms per molecule		n _D ²⁰	Vapor pressure (mm., 20°C.)
		Found	Required		
Ethyl acetate	CH ₃ COOCH ₂ CH ₃	—	—	1.3723	72.9
Ethyl-1-H ₂ ² acetate	CH ₃ COOCH ₂ ² CH ₃	1.98	2	1.3718	74.2
Ethyl-2-H ₃ ² acetate	CH ₃ COOCH ₂ CH ₃ ²	2.97	3	1.3716	74.0
Ethyl acetate-H ₃ ²	CH ₃ ² COOCH ₂ CH ₃	2.97	3	1.3712	74.8
Ethyl-H ₃ ² acetate	CH ₃ COOCH ₂ ² CH ₃ ²	4.97	5	1.3710	75.0
Ethyl-1-H ₂ ² acetate-H ₃ ²	CH ₃ COOCH ₂ ² CH ₃ ²	4.97	5	1.3707	75.3
Ethyl-2-H ₃ ² acetate-H ₃ ²	CH ₃ ² COOCH ₂ CH ₃ ²	5.92	6	1.3704	75.9
Ethyl-H ₃ ² acetate-H ₃ ²	CH ₃ ² COOCH ₂ ² CH ₃ ²	7.94	8	1.3700	76.3

METHYL ACETOACETATE-4-H₁²

J. R. Johnson and V. J. Shiner, Jr., J. Am. Chem. Soc., 75, 1350 (1953).

A. Procedure

The reaction is carried out in a 25-ml. 3-necked flask. The materials are added through one neck by use of a small dropping funnel, which can be weighed before and after addition. A stream of nitrogen is bubbled through the solution by means of a capillary inlet in the second neck. In the third neck is placed a 20-cm., jacketed, glass-helix packed tube which can be used as a reflux condenser or as a distilling column.

A mixture of 8.70 g. (0.104 mole) of ketene dimer and 8.02 g. (0.252 mole) of methanol-H₂² is refluxed for 85.5 hours. The mixture is heated in an oil-bath at 78–82°. In fractionation of the reaction mixture, methanol-H₂² is distilled at 64–65° (740 mm.). Then the pressure is reduced, and 2.5 ml. of diketene, b.p. 55–58° (60 mm.), is collected; n_D²⁰ 1.4280. The pressure is again lowered and 3.5 ml. of methyl acetoacetate-2-H₂²-4-H₁², b.p. 77–78° (38 mm.), n_D²⁰ 1.4188, is obtained. The ester is refluxed for 2 hours with 15.5 molar equivalents of methanol which is then distilled off, and the process is repeated with more methanol (Note 1). The latter is also removed and the ester is distilled (Note 2).

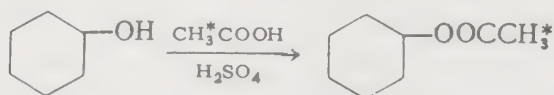
B. Notes

1. The diketene and the methyl acetoacetate contained 0.482 and 1.398 atoms of deuterium per molecule, respectively. Two exchanges of the ester with ordinary methanol removed the exchangeable deuterium in the

2-position and reduced the deuterium content to 0.403 atom of deuterium per molecule.

2. From their experimental evidence, of which this is only a part, Johnson and Shiner have concluded that ketene dimer is a single molecular species to be represented as 3-butenic β -lactone.

CYCLOHEXYL ACETATE- H_3^2

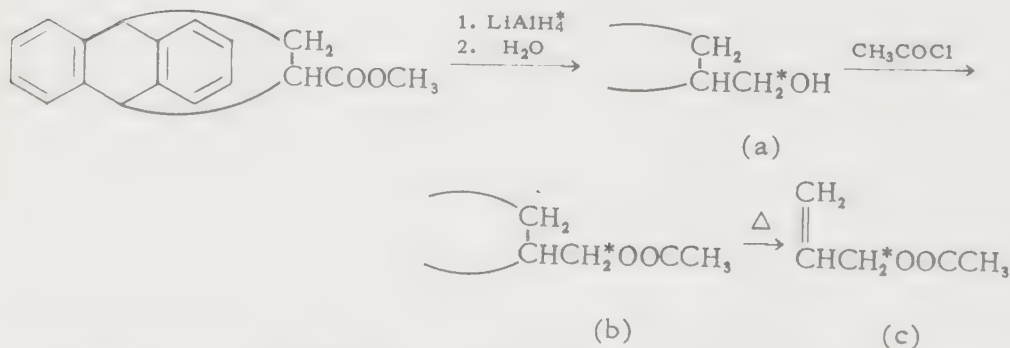


B. Nolin and R. N. Jones, *Can. J. Chem.*, 30, 727 (1952)

Procedure

A mixture of 2.0 ml. of freshly distilled cyclohexanol, 2.5 ml. of acetic- H_3^2 acid and 0.13 ml. of concentrated sulfuric acid is refluxed for 1 hour. The reaction mixture is cooled, diluted with water and extracted with ether. The ethereal solution is washed with aqueous sodium hydroxide and water and dried over sodium sulfate. After removal of ether the residue is fractionally distilled under reduced pressure, b.p. 174.2–174.8°; n_D^{20} 1.4413.

ALLYL-1- H_2^2 ACETATE



P. D. Bartlett and F. A. Tate, *J. Am. Chem. Soc.*, 75, 91 (1953).

A. Procedure

(a) 9, 10-Dihydro-9, 10-ethanoanthracene-11-methanol- α - H_2^2 (Note 1). In a 1-l. flask equipped with stirrer, reflux condenser and drying tube are placed 1 g. (0.024 mole) of lithium aluminum hydride- H_4^2 (Note 2) and 600 ml. of dry tetrahydrofuran. While the mixture is refluxed, 9.29 g. (0.0352 mole) of methyl 9,10-dihydro-9,10-ethanoanthracene-11-carboxylate is

added slowly with vigorous stirring over a 2-hour period. The mixture is refluxed with stirring for 10-12 hours and then hydrolyzed with 10% sulfuric acid. The tetrahydrofuran is removed near room temperature under vacuum. The residue is extracted four times with a total of 600 ml. of ether. The combined ether solution is washed with sodium carbonate solution, dried over potassium carbonate, then concentrated and decolorized. After crystallization and recrystallization from an ether-petroleum ether mixture, the product melts at 110° (Note 3).

(b) *9,10-Dihydro-9,10-ethanoanthracene-11-methyl- α -H₂² Acetate*. 9,10-dihydro-9,10-ethanoanthracene-11-methanol- α -H₂², 5.82 g. (0.024 mole), is dissolved in 50 ml. of acetone. The solution is refluxed, and 10 g. (0.127 mole) of acetyl chloride is added through the condenser. After refluxing for 15 minutes, the yellow solution is poured over 800 ml. of ice, and the reactor is rinsed with acetone. The product, a white amorphous mass, is broken up, filtered and dried at 40° . After treatment with carbon, crystallization and recrystallization from ether, the product melts at 121° ; yield, 95%.

(c) *Allyl-1-H₂² Acetate*. In a 150-ml. pear-shaped flask, equipped with a Vigreux column (insulated with glass wool) and a thermometer, is placed 7.54 g. (0.027 mole) of 9,10-dihydro-9,10-ethanoanthracene-11-methyl- α -H₂² acetate. A Wood's metal-bath at 350 - 360° is raised just above the level of the melted ester. In 3-4 minutes, the allyl-1-H₂² acetate begins to distill, at a vapor temperature of 103° . The pyrolysis is discontinued when the rate of distillation has dropped to 0.2 of the maximum rate. The yield of allyl-1-H₂² acetate is 88.2% (Note 4).

B. Notes

1. Attempts to reduce acrylic acid, its esters or its salts directly, in a manner suitable for the preparation of allyl-1-H₂² acetate, failed because of accompanying polymerization. Bartlett and Tate, therefore, protected the double bond during reduction by the addition of methyl acrylate to anthracene to form methyl 9,10-dihydro-9,10-ethanoanthracene-11-carboxylate.

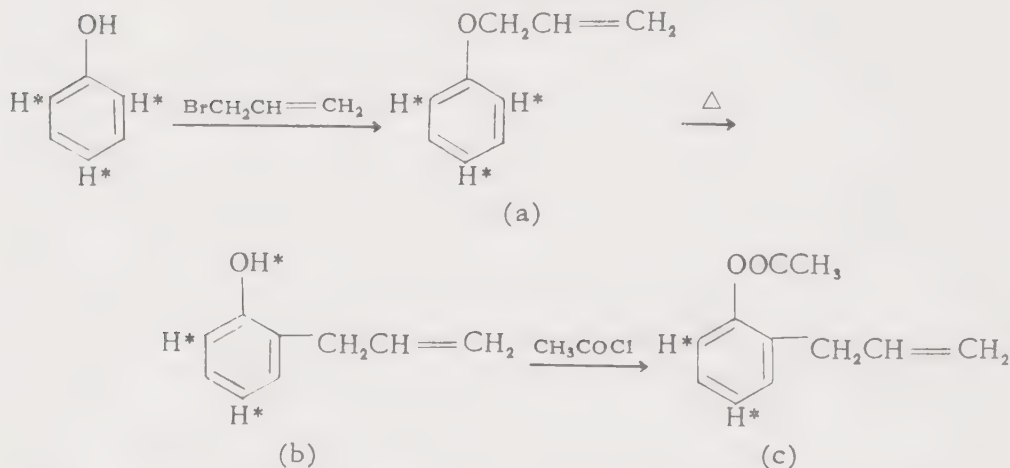
2. The lithium aluminum hydride-H₂², obtained from Metal Hydrides, Inc., Beverly, Mass., assayed 92.5% active, and 99.8% of its hydrogen was deuterium.

3. The best yield was 85.7% based on the ester used. Attempts to diminish the excess of the lithium aluminum hydride did not improve its utilization.

4. The allyl-1-H₂² acetate was used in polymerization experiments and compared with ordinary allyl acetate. There was substantially more polymerization for a given amount of benzoyl peroxide initiation in the deuterated than in the normal monomer. In a general way the results

confirm that a 1-hydrogen atom of the allyl group is transferred in the chain-terminating step in the polymerization of allyl acetate. Assuming that all chains are terminated in this way, hydrogen is estimated to be transferred in this atomic displacement reaction about three times as fast as deuterium. Chain transfer in the polymerization of allyl acetate must also proceed through the 1-acetoxyallyl free radical.

2-ALLYLPHENYL-4,6- H^2 ACETATE



G. B. Kistiakowsky and R. L. Tichenor, *J. Am. Chem. Soc.*, **64**, 2302 (1942).

A. Procedure

(a) *Allyl Phenyl-2,4,6- H^2_3 Ether*. Into a 250-ml. 3-necked flask, equipped with a wire stirrer, reflux condenser and dropping funnel, are quickly added in this order, 13.5 g. (0.34 mole) of sodium hydroxide, 25 ml. of water, 15 ml. of freshly distilled allyl bromide, 25 ml. of acetone and 10 g. (0.106 mole) of phenol-2,4,6- H^2_3 . The mixture is stirred and heated under reflux for 2 hours, cooled, and extracted with petroleum ether. The extract is washed with sodium hydroxide, then with water and dried over magnesium sulfate (Note 1). After concentration by distillation on a steam-bath, the allyl phenyl-2,4,6- H^2_3 ether is distilled at a pressure of 10 mm. through a small Podbielniak column (Note 2).

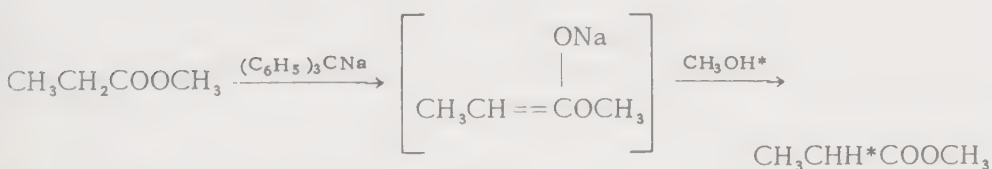
(b) *2-Allylphenol- H^2 -4,6- H^2_2* . For the Claisen rearrangement, the purified ether is placed in a clean Pyrex tube, cooled, evacuated, frozen, and melted under vacuum to remove dissolved gases. The tube is sealed and heated in a Wood's metal bath until samples of the nonisotopic ether, rearranging simultaneously, are found to be almost entirely soluble in 20% sodium hydroxide solution. At a bath temperature of 230–240°, the allyl phenyl ether is practically all rearranged in 5–6 hours.

(c) *2-Allylphenyl-4,6-H₂² Acetate*. The acetate is prepared by heating 3-4 g. of the 2-allylphenol-H²-4,6-H₂² with 3 ml. of acetyl chloride, under reflux, until evolution of hydrogen chloride ceases (Note 3). After removal of excess acetyl chloride and recrystallization, the physical constants of the 2-allylphenyl-4,6-H₂² acetate agree with those in the literature.

B. Notes

1. A neutral drying agent is used to minimize exchange of deuterium.
2. The product was colorless, and the boiling point and refractive index agreed with those in the literature.
3. The evolved hydrogen and deuterium chlorides were trapped, freed of acetyl chloride and assayed for deuterium to establish that the *ortho*-deuterium had migrated to the oxygen during rearrangement.

METHYL PROPIONATE-2-H₁²



K. B. Wiberg, J. Am. Chem. Soc., 77, 5987 (1955).

A. Procedure

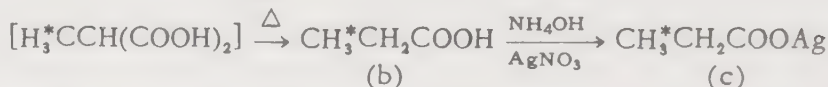
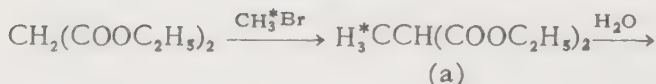
To 100 ml. of a 0.45 *N* solution of triphenylmethylsodium¹ in ether is added 3 ml. (0.021 mole) of methyl propionate, with shaking (Note 1). This solution is added, with stirring, to 15 ml. of methanol-H² mixed with 20 ml. of butyl ether. After one-half minute, 55 ml. (20% excess) of 1 *N* hydrochloric acid is added rapidly, with stirring. The mixture is transferred to a separatory funnel, and the aqueous layer is discarded. The ether solution is washed with water and dried over anhydrous copper sulfate. After removal of ether, distillation of the residue yields 1.5 ml. of methyl propionate-2-H₁², b.p. 77-79° (Note 2).

B. Notes

1. A similar use of triphenylmethylsodium is described by Hauser and Renfrew.²
2. The deuterium isotope effect in this reaction, $k\text{H}/k\text{H}^2$, was 1.16.

¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 607.

²*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 268.

SILVER PROPIONATE-3- H_3^2 

B. Nolin, Can. J. Chem., 31, 1257 (1953).

A. Procedure

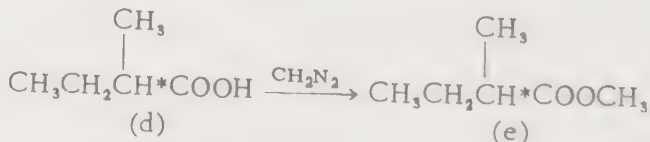
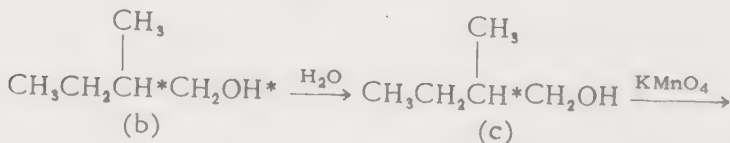
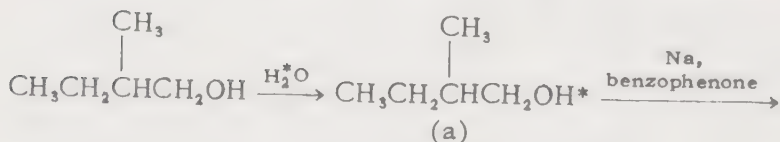
(a) *Ethyl Methyl- H_3^2 -malonate*. Ethyl malonate is alkylated with methyl- H_3^2 bromide essentially according to the procedure of Weiner.¹

(b) *Propionic-3- H_3^2 Acid*. Ethyl 2-methyl- H_3^2 -malonate is hydrolyzed, and the resulting acid is decarboxylated to obtain propionic-3- H_3^2 acid.

(c) *Silver Propionate-3- H_3^2* . According to the procedure of Nolin and Leitch² (see silver acetate- H_3^2), a cold aqueous solution of the acid is neutralized carefully with cold dilute ammonia. Silver propionate-3- H_3^2 is precipitated by the addition of silver nitrate solution. The silver salt is collected, washed with cold water and dried. The yield of the silver salt is nearly quantitative; product remaining in the filtrate is recovered by concentration.

¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1948, p. 279.

²B. Nolin and L. C. Leitch, Can. J. Chem., 31, 153 (1953).

METHYL 2-METHYLBUTYRATE-2- H^2 

W. v. E. Doering and T. C. Aschner, J. Am. Chem. Soc., 75, 393 (1953).

A. Procedure (Note 1)

(a) (-)-2-Methyl-1-butanol- H^2 . A mixture of 22 g. of (-)-2-methyl-1-butanol and 3.0 g. of water- H_2^2 is refluxed for 16 hours under a nitrogen at-

mosphere. (-)-2-Methyl-1-butanol- H^2 , 20.0 g., b.p. 128° , $[\alpha]_D^{27} -5.81^\circ$, is isolated by distillation.

(b) 2-Methyl-1-butanol- H^2 -2- H^2 . (-)-2-Methyl-1-butanol- H^2 , above, is racemized by heating at 120° , for 8 hours under nitrogen, with 5 mole per cent each of sodium and benzophenone. Completely racemized 2-methyl-1-butanol- H^2 -2- H^2 , 12.5 g., is recovered by distillation.

(c) 2-Methylbutanol-2- H^2 . The above 12.5 g. of 2-methyl-1-butanol- H^2 -2- H^2 is refluxed with 400 ml. of water for 23 hours. Then 12.0 g. of 2-methyl-1-butanol-2- H^2 is recovered by distillation.

(d) 2-Methylbutyric-2- H^2 Acid. A solution of 5.0 g. of 2-methyl-1-butanol-2- H^2 , 13 g. of potassium permanganate and 1.5 g. of potassium hydroxide in 250 ml. of water is stirred for 40 minutes. Then the solution is extracted with ether, acidified with hydrochloric acid and extracted with chloroform. The chloroform extract is washed with 50 ml. of water and dried over magnesium sulfate. After removal of chloroform, fractionation of the residue through a 10-cm. Vigreux column gives 3.0 g. of racemic 2-methyl-butyric-2- H^2 acid, b.p. 173 – 174° (Note 2).

(e) Methyl 2-Methylbutyrate-2- H^2 . To 1.6 g. of the above acid is added an excess of diazomethane in ether. The ether is removed, and the product is fractionally distilled to obtain 0.6 g. of the methyl ester, b.p. 110 – 116° .

B. Notes

1. The stereochemical equilibrium of carbinols is catalyzed by aluminum alkoxides as well as by alkali alkoxides, requires initiation by a carbonyl compound, and proceeds by an oxidation-reduction mechanism.^{1,2} The oxidation-reduction involves a pair of carbinol-carbonyl systems, between which establishment of equilibrium is accelerated by aluminum alkoxide in the Meerwein-Ponndorf-Verley reduction³ and Oppenauer oxidation,⁴ and may also be catalyzed by alkoxide ion.^{1,2}

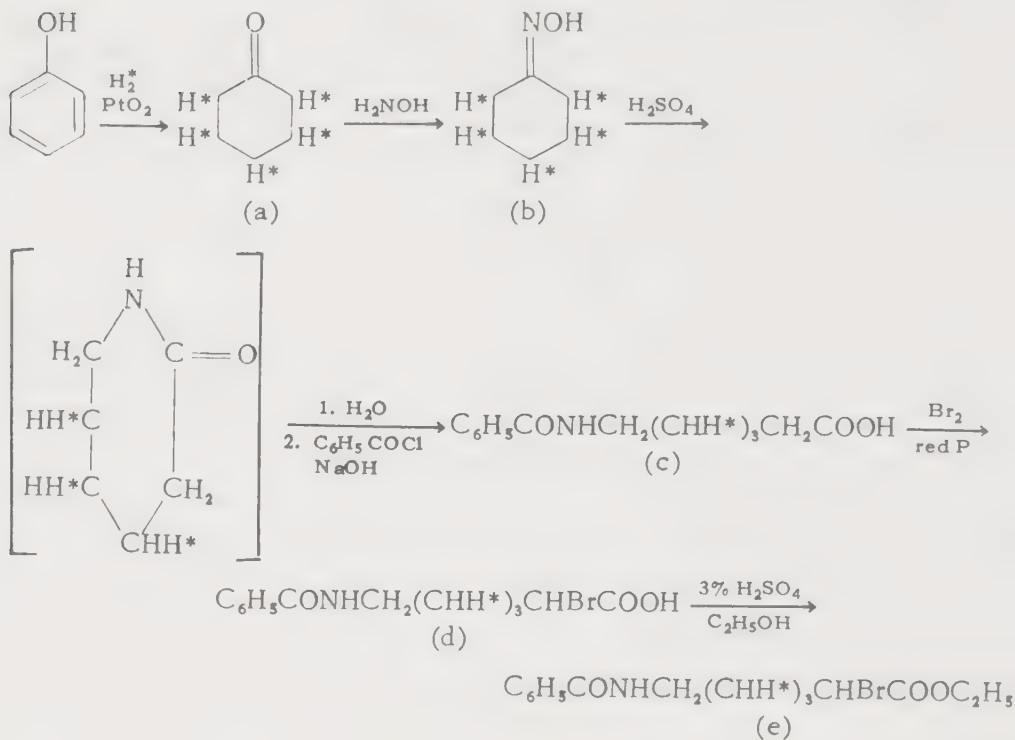
2. By a procedure similar to that described, 15.0 g. of (-)-2-methyl-1-butanol- H^2 was heated at 120° for 40 hours, with 5 mole per cent each of aluminum isopropoxide and benzophenone. The carbinol was isolated, refluxed with 400 ml. of water for 24 hours and purified by distillation to obtain 10.0 g. of (-)-2-methyl-1-butanol-2- H^2 . Oxidation of 5 g. of this material with alkaline permanganate gave 2.7 g. of 2-methylbutyric-2- H^2 acid, b.p. 173 – 174° , $[\alpha]_D^{27} +10.4^\circ$ (methanol).

¹W. v. E. Doering, G. Cortez and L. H. Knox, J. Am. Chem. Soc., 69, 1700 (1947).

²W. v. E. Doering, and T. C. Aschner, *ibid.*, 71, 838 (1949).

³Organic Reactions, Vol. II, Wiley, New York, 1944, p. 178.

⁴T. Bersin, *Newer Preparative Methods of Organic Chemistry*, Interscience Publishers, Inc., New York, 1948, p. 125.

ETHYL 6-BENZAMIDO-2-BROMOHEXANOATE-3,4,5- H_3^2 

N. Weissman and R. Schoenheimer, J. Biol. Chem., 140, 779 (1941).

A. Procedure

(a) *Cyclohexanone-2,3,4,5,6- H_3^2* . Redistilled phenol, 70 g., and 0.71 g. of platinum oxide catalyst are placed in a steam-jacketed flask which is part of a hydrogenation apparatus. The system is filled with a mixture of equal volumes of hydrogen and deuterium; the flask is heated by steam, and shaking is started (Note 1). When 28 l. of hydrogen has been consumed (Note 2), the reaction is interrupted, ether is added, and the catalyst is filtered off. The filtrate is extracted with 10% sodium hydroxide solution to remove unchanged phenol and dried, and the ether is removed.

(b) *Cyclohexanone-2,3,4,5,6- H_3^2 Oxime*. The oxime of the crude cyclohexanone-2,3,4,5,6- H_3^2 is prepared according to the procedure of Eck and Marvel.¹ The yield of crude oxime is 49 g.

(c) *6-Benzamidohexanoic-3,4,5- H_3^2 Acid*. Rearrangement of the cyclohexanone oxime and hydrolysis of the resulting lactam are also according to Eck and Marvel¹ (Note 3), as in the following example. To 10 g. of the oxime in a 300-ml. Erlenmeyer flask is added 20 ml. of sulfuric acid (Note 4). The mixture is heated over a low flame until the violent reaction, which lasts a few seconds, has subsided. The acid solutions

from 5 such reactions are combined in a 2-l. flask and diluted with 1.25 l. of water. In order to hydrolyze the lactam and to decolorize the solution, it is gently boiled for $1\frac{1}{2}$ hours with about 5 g. of activated carbon (Note 5). Then, the solution is filtered and made neutral to litmus with 50% sodium hydroxide solution. The solution is again decolorized, and the filtrate, in a 3-l. flask equipped with a mechanical stirrer and cooled in an ice-bath, is made alkaline by the addition of 27.5 g. of sodium hydroxide dissolved in water. To the well-stirred solution kept at a temperature of about 10° , 47 g. of benzoyl chloride is added dropwise from a separatory funnel. After the addition is complete, the mixture is stirred for about one hour and then filtered. The cold filtrate is slowly acidified to Congo red by the addition of dilute hydrochloric acid (10%). The product is collected on a suction filter and when dry is washed with two 50-ml. portions of petroleum ether to remove any benzoic acid. After drying in a vacuum desiccator over sulfuric acid, the yield of product, m.p. $77-80^{\circ}$, is 150 g. (72%, based on the oxime).

(d) *6-Benzamido-2-bromohexanoic-3,4,5- H_3^2 Acid*. The bromination of 6-benzamidohexanoic-3,4,5- H_3^2 acid is done according to the following procedure described by Eck and Marvel.¹ An intimate mixture of 150 g. of the dry acid and 26.4 g. of dry red phosphorus is placed in a 1-l. 3-necked flask. The latter is equipped with a separatory funnel, an air condenser which is connected through a calcium chloride tube to a water trap, and a mechanical stirrer (Note 6). The reaction flask is placed in an ice-salt bath, and to the cold, stirred mixture 408 g. of dry bromine is added dropwise from the separatory funnel. When all of the bromine is added, the cooling bath is removed, and the reaction mixture is slowly warmed and finally heated in a boiling water-bath until the bromine vapors practically disappear. After the mixture is poured onto cracked ice in a 1-l. beaker, any excess bromine is reduced with a slow stream of sulfur dioxide, and material remaining in the reaction flask is treated in the same manner. The solid product is collected on a suction filter, washed with three 50-ml. portions of water and air-dried. The yield of 2-bromo acid, m.p. $163-166^{\circ}$, is 180 g. (90%).

(e) *Ethyl 6-Benzamido-2-bromohexanoate-3,4,5- H_3^2* . The above 2-bromo acid is refluxed for 3 hours with 3% sulfuric acid in ethanol. The resulting ester, which is insoluble in ligroin and water, is very soluble in methanol, ethanol, acetone, benzene, chloroform and ethyl acetate, and slightly soluble in petroleum ether. After recrystallization from a mixture of benzene and ligroin, the product melts at $56-57^{\circ}$.

B. Notes

1. Ethanol was not used as a solvent because of exchangeable hydrogen, and the apparatus was not adaptable to high pressure or vapor phase

hydrogenation. Therefore, molten phenol was hydrogenated at 95° under one atmosphere pressure.

2. The reaction slowed after about half the theoretical amount of hydrogen had been consumed but was revived by the addition of 350 mg. of catalyst.

3. The rearrangement of the oxime was carried out in 10-gram portions, as the reaction was violent.

4. The sulfuric acid, sp. gr. 1.783, was prepared by mixing 5 volumes of concentrated sulfuric acid and 1 volume of water.

5. During the rearrangement and hydrolysis step, the deuterium originally present in the 2 and 6 positions of the ketone is presumably removed by exchange with the solvent.

6. The stirrer must be quite powerful because the mixture becomes very viscous during the reaction.

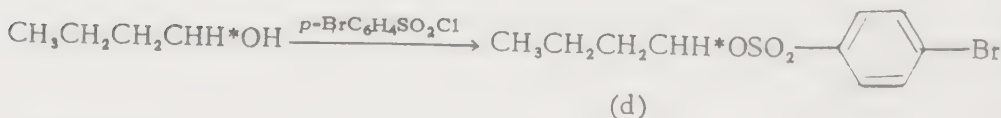
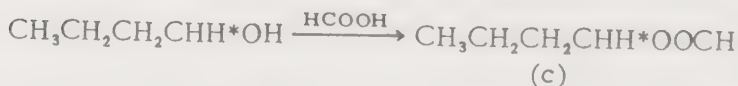
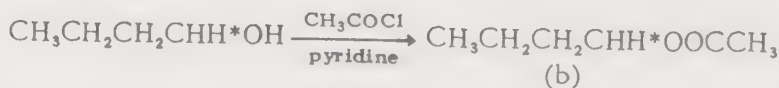
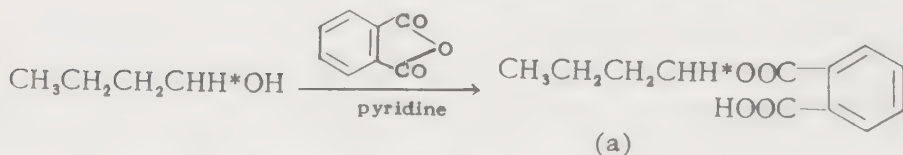
C. Other Preparations

Deuterium-labeled cyclohexanone has also been prepared by hydrogen exchange with water- H_2^2 in the presence of a platinum catalyst.² Hydrogenation also occurred, and the product was predominantly H^2 -cyclohexanol.

¹J. E. Eck and C. S. Marvel, *J. Biol. Chem.*, **106**, 387 (1934); *Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 76.

²N. Weissman and R. Schoenheimer, *J. Biol. Chem.*, **140**, 779 (1941).

BUTYL-1- H_1^2 *p*-BROMOBENZENESULFONATE



A. Streitwieser, Jr., *J. Am. Chem. Soc.*, **77**, 1117 (1955).

A. Procedure (Note 1)

(a) *Butyl-1- H_1^2 Hydrogen Phthalate.* A mixture of 9.7 ml. of butanol-1- H_1^2 and 21 g. of phthalic anhydride in 100 ml. of dry pyridine is refluxed

overnight. The solution is poured into a mixture of ice and dilute hydrochloric acid, and the initial oily precipitate solidifies. Recrystallization of the product from benzene-hexane gives 18.1 g. of crystals which are recrystallized a second time from the same solvent. The yield of large, colorless prismatic crystals is 14.2 g.; $[\alpha]_D^{25} + 0.070^\circ$; $\alpha_D + 0.069 \pm 0.004^\circ$ ($l = 4$), $+ 0.036 \pm 0.004^\circ$ ($l = 2$, $c = 25.0$, acetone) (Note 2).

(b) *Butyl-1-H₁² Acetate*. A solution of 2.5 ml. of acetyl chloride in 5 ml. of pentane is added dropwise, with stirring, to a mixture of 2.5 ml. of butanol-1-H₁², 5 ml. of pyridine and 12 ml. of pentane which is cooled in an ice-bath. After the reaction mixture is stirred for several hours at room temperature, the pentane solution is washed with dilute acid and with water. After the solution is dried it is distilled through a Vigreux column. A second distillation of the center cut yields 2.2 ml., b.p. 123–125°; $\alpha_D^{25} + 0.332 \pm 0.005^\circ$, $+ 0.335 \pm 0.006^\circ$, $\alpha_{5461}^{25} + 0.395 \pm 0.004^\circ$ ($l = 4$), $\alpha_D^{25} + 0.168 \pm 0.005^\circ$, $\alpha_{5461}^{25} + 0.198 \pm 0.007^\circ$ ($l = 2$).

(c) *Butyl-1-H₁² Formate*. A solution of 3 ml. of butanol-1-H₁² in 3.0 ml. of 98% formic acid is refluxed for 24 hours. The mixture is cooled, diluted with water and extracted with pentane. The pentane solution is washed with water and dried, and is then fractionated through a Vigreux column. The product is redistilled to obtain 1.2 ml. of butyl-1-H₁² formate, b.p. 105–105.5°; $\alpha_D^{25} + 0.213 \pm 0.005^\circ$, $+ 0.218 \pm 0.007^\circ$, $+ 0.218 \pm 0.004^\circ$; $\alpha_{5461}^{25} + 0.259 \pm 0.007^\circ$, $+ 0.247 \pm 0.005^\circ$ ($l = 2$).

(d) *Butyl-1-H₁² p-Bromobenzenesulfonate*. A mixture of 5.0 ml. of butanol-1-H₁² and 45 ml. of pyridine is cooled in an ice salt-bath for several minutes; then 16.0 g. of *p*-bromobenzenesulfonyl chloride is added. After the reaction mixture is stirred magnetically for 1 hour, in the cooling bath, it is poured into a mixture of 56 ml. of concentrated hydrochloric acid and 225 ml. of ice and water. The resulting heavy oil is extracted into 110 ml. of hexane, and the solution is dried. The dry solution is filtered and cooled in a Dry Ice-bath. The yield of butyl-1-H₁² *p*-bromobenzenesulfonate, which precipitates as a faintly cloudy oil ($n_D^{21} 1.5322$), is 14 g. (88%) (Note 3).

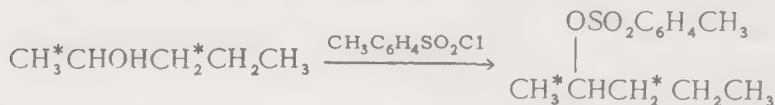
B. Notes

1. Optical rotations were taken on a Rudolph precision polarimeter on which readings could be made to the nearest 0.001° . With the exception of butyl-1-H₁² hydrogen phthalate which is solid, determinations of rotation were made on the pure liquids. A highly purified sample of butanol-1-H₁² had the following properties: b.p. 116–117°, $d_4^{25} 0.8169$, $x_D^{22} - 0.117 \pm 0.005^\circ$, $\alpha_{5461}^{22} - 0.143 \pm 0.007^\circ$ ($l = 4$).

2. After the sixth recrystallization of the product, 6.4 g. was obtained, having $[\alpha]_D^{25} + 0.073^\circ$; $\alpha_D + 0.068 \pm 0.008^\circ$ ($l = 4$), $+ 0.040 \pm 0.006^\circ$ ($l = 2$, c , 25.0, acetone).

3. The product was centrifuged before the rotation was determined, but the material was still colored sufficiently to prevent the use of polarimeter tubes more than 1 dc. in length; $\alpha_D^{25} + 0.020 \pm 0.005^\circ$, $+ 0.022 \pm 0.002^\circ$, $\alpha_{5461} + 0.021 \pm 0.007^\circ$, $+ 0.008 \pm 0.005^\circ$ ($l = 1$).

1-METHYL- H_3^2 -BUTYL-2- H_2^2 *p*-TOLUENESULFONATE



E. S. Lewis and C. E. Boozer, J. Am. Chem. Soc., 76, 791 (1954).

A. Procedure

1-Methyl- H_3^2 -butyl-2- H_2^2 *p*-toluenesulfonate is prepared in 30% yield by adaptation of the following procedure of Cram.¹ A solution of 0.25 mole of the alcohol in 530 ml. of pure, dry pyridine is cooled to -7° and treated with 0.27 mole of *p*-toluenesulfonyl chloride. The latter is added in small portions during several hours such that the temperature of the solution remains below 0° . After the addition is complete, the mixture is stored at 0° for 3 weeks (Note 1). The mixture is then poured into a mixture of ice and water. The solid which separates is collected, the filtrate is extracted with benzene, and the solid is dissolved in the benzene solution. The resulting solution is washed successively with water, ice-cold 1 *N* sulfuric acid, water, and sodium carbonate solution. The benzene solution is then dried and evaporated under partial vacuum (20 mm.) at room temperature (Note 2).

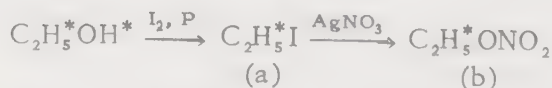
B. Notes

1. The yield of *p*-toluenesulfonate ester was doubled by storing the pyridine solution for 3 weeks instead of 1 week.

2. The product did not crystallize when the solvent was removed, but analysis by solvolysis indicated a purity of 98–99%.

¹D. J. Cram and F. A. A. Elhafez, J. Am. Chem. Soc., 74, 5851 (1952).

ETHYL- H_5^2 NITRATE



R. Steinberger, C. A. Orlick and V. P. Schaaf, J. Am. Chem. Soc., 77, 4748 (1955).

A. Procedure

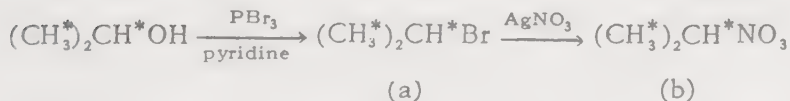
(a) *Iodoethane-H₅²*. A mixture of 0.5 g. (16 meq.) of red phosphorus, 1.66 g. (31.9 mmoles) of ethanol-H₆² and 4.3 g. (33.9 meq.) of resublimed iodine is refluxed for 2.5 hours. The iodoethane-H₅² is distilled into a cooled flask containing 1.5 g. of potassium carbonate and a small amount of mercury (Note 1).

(b) *Ethyl-H₅² Nitrate*. Then, while the flask is cooled in an ice-bath, 8.5 g. of silver nitrate (50 mmoles) is added slowly. After the mixture is refluxed for 2 hours, the crude ethyl-H₅² nitrate is distilled into a flask containing 1.5 g. of potassium carbonate and a droplet of mercury. A small amount of calcium sulfate is added to remove cloudiness (Note 2). An additional 7.5 g. (44 mmoles) of silver nitrate is added, and the mixture is again refluxed for 2 hours. The product is distilled into a cold receiver equipped with a drying tube. The yield of ethyl-H₅² nitrate, b.p. 87.8–88.0° (cor.), is 2.41 g. (83%); n_D^{25} 1.3807.

B. Notes

1. The potassium carbonate neutralizes any acid, and the mercury removes excess iodine.

2. This is presumably water.

1-METHYLETHYL-H₇² NITRATE

R. Steinberger, C. A. Orlick and V. P. Schaaf, J. Am. Chem. Soc., 77, 4748 (1955).

Procedure

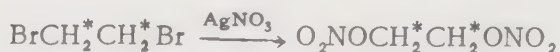
(a) *2-Bromopropane-H₇²*. To a mixture of 5 g. (83 mmoles) of 2-propanol-1,2,3-H₇² and 1 g. (13 mmoles) of pyridine, chilled in an ice-bath, is added dropwise 10–11 g. (111–122 meq.) of phosphorus tribromide. The mixture is refluxed until fumes of hydrogen bromide cease to be evolved (1–2 hours). When the product is distilled, washed and dried, the yield of 2-bromopropane-H₇² is 68–73%.

(b) *1-Methylethyl-H₇² Nitrate*. To the 2-bromopropane-H₇², cooled in an ice-bath, finely pulverized silver nitrate is added very slowly in 75–100% excess. When the addition is completed, the mixture is warmed slowly and then distilled at reduced pressure (about 100 mm.). A small amount of silver nitrate is placed in the receiver, and a second distillation is

carried out to obtain a pure product. The b.p. of the product is 99.7° ; n_D^{25} 1.3849, d_4^{20} 1.095.

2-Bromopropane-2- H^2 , 1-methylethyl-1- H^2 nitrate (b.p. 101.2° , n_D^{25} 1.3876, d_4^{20} 1.047), 2-bromopropane-1,3- H_6^2 and 1-methyl- H_3^2 -ethyl-2- H_3^2 nitrate (b.p. 100.4° , n_D^{25} 1.3854, d_4^{20} 1.098) are prepared according to the above procedure from the corresponding alcohols.

ETHYLENE- H_4^2 NITRATE



R. Steinberger, C. A. Orlick and V. P. Schaaf, J. Am. Chem. Soc., 77, 4748 (1955).

A. Procedure

Ethylene- H_4^2 bromide, 10 g. (52 mmoles), is added to a solution of 35 g. (206 mmoles) of silver nitrate in 50 ml. of acetonitrile (Note 1). The solution is heated at $70-75^{\circ}$ for 2 hours. The solution is filtered to remove silver bromide which is washed with fresh solvent. The solvent is evaporated to obtain 7.18 g. (88.5%) of ethylene- H_4^2 nitrate, n_D^{24} 1.4445 (Note 2).

B. Notes

1. The method of Ferris¹ was used.
2. The product was used without further distillation since, in preliminary runs, the purity was shown to be equal to that of a distilled sample.

¹A. F. Ferris, K. W. McLean, I. G. Marks and W. D. Emmons, J. Am. Chem. Soc., 75, 4078 (1953).

BIS(1-METHYLETHYL-1- H^2) CHROMATE



A. Leo and F. H. Westheimer, J. Am. Chem. Soc., 74, 4383 (1952).

A. Procedure

(a) 2-Propanol-2- H^2 . Isotopically pure 2-propanol-2- H^2 is prepared by the reduction of 30 g. of acetone with a suspension of 4 g. of lithium aluminum hydride- H_4^2 in 150 ml. of ether, under a nitrogen atmosphere (see Brown¹). When the reaction is complete, the mixture is treated with

430 ml. of 6% sulfuric acid. The resulting mixture is then fractionated through an 18-inch Podbielniak column at a reflux ratio of 25 to 1. The aqueous azeotrope of 2-propanol-2-H², 17.4 g., which boils at 79.0–80.5°, is refluxed over calcium oxide, and the dry alcohol is distilled through a small tantalum-wire column. A fraction of pure 2-propanol-2-H², 12.1 g. (53%), is collected; b.p. 82.2° (750 mm.) (Note 1).

(b) *Bis(1-methylethyl-1-H²) Chromate* (Note 2). A method of preparing this ester, which avoids the use of water, consists of shaking a benzene solution of 2-propanol-2-H² with a few crystals of chromic oxide. The concentration of ester is determined by either analysis of the solution for chromium or spectroscopic analysis for the ester (Note 3).

B. Notes

1. Oxidation of the product to acetone by the Oppenauer method^{2,3} and analysis of the latter for excess deuterium showed that no exchange of hydrogen in the methyl groups had accompanied the reduction with lithium aluminum hydride-H₄².

2. The preparation of solutions of this ester in benzene or toluene have been described.⁴

3. Dependent upon experimental conditions, isopropyl chromate, in benzene solution, can undergo either hydrolysis or internal oxidation-reduction. The rate of the latter reaction is approximately first order with respect to pyridine, and is first order with respect to the chromic acid ester, but the rate is decreased by the addition of isopropyl alcohol. The rate of internal oxidation-reduction of bis(1-methylethyl-1-H²) chromate is only about one-fifth as fast as the corresponding rate for isopropyl chromate.

C. Other Preparations

2-Propanol-2-H² has been prepared⁵ by the reduction of acetone with lithium aluminum hydride-H₄², similarly to the procedure described, and by exchange^{2,6} of the hydroxyl hydrogen-H² of 2-propanol-H²-2-H² with water.

2-Propanol-H²-2-H² has been prepared^{2,5-7} by the catalytic hydrogenation of acetone with hydrogen-H₂² and an iron-promoted platinum-on-carbon catalyst.⁸ Friedman and Turkevitch⁷ obtained mass spectrometric data showing that the predominant product of the reduction is (CH₃)₂CH²OH². Their data indicated a small amount of exchange in the methyl groups. Reduction of acetone with hydrogen-H₂² and several metallic catalysts (platinum, nickel and copper chromite) has been studied.⁹ The results at 25° indicated that the acetone molecules reduced were almost entirely in the keto-form; whereas at 200°, considerable addition of hydrogen-H₂² to the ethylenic bond of the enol-form occurred.

Friedman and Turkevitch⁶ have prepared 2-propanol-H² by the hydrolysis of aluminum isopropoxide with water-H².

¹*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 469.

²F. Westheimer and N. Nicolaides, *J. Am. Chem. Soc.*, 71, 25 (1949).

³R. Baker and H. Adkins, *ibid.*, 62, 3305 (1940).

⁴F. Holloway, M. Cohen and F. Westheimer, *ibid.*, 73, 65 (1951).

⁵E. D. Williams, K. A. Krieger and A. R. Day, *ibid.*, 75, 2404 (1953).

⁶L. Friedman and J. Turkevitch, *ibid.*, 74, 1666 (1952).

⁷*Idem*, 74, 1669 (1952).

⁸*Organic Syntheses*, Coll. Vol. I, 2nd. ed., Wiley, New York, 1941, p. 469.

⁹L. C. Anderson and N. W. MacNaughton, *J. Am. Chem. Soc.*, 64, 1456 (1942).

HYDROGEN-H² CYANIDE AND HYDROGEN-H² CYANIDE-C¹³

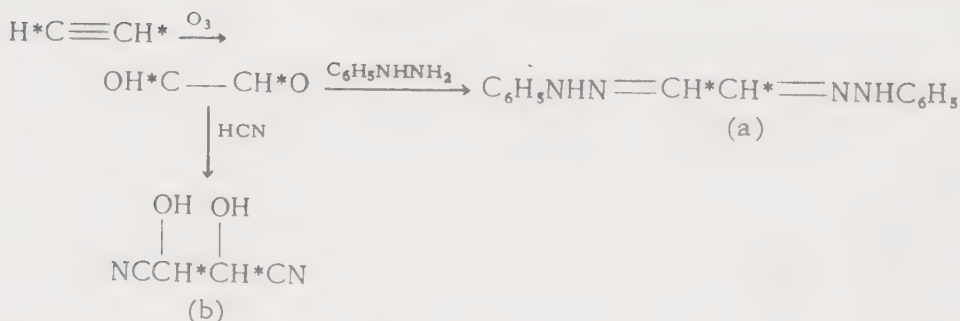


W. S. Richardson, *J. Chem. Phys.*, 19, 1213 (1951); G. E. Hyde and D. F. Hornig, *ibid.*, 20, 647 (1952).

Procedure

Solid potassium cyanide is added to a syrupy mixture of water-H² and phosphorus pentoxide. The evolved hydrogen-H² cyanide is collected in a Dry Ice-cooled trap. Potassium cyanide-C¹³ is used in the preparation of hydrogen-H² cyanide-C¹³.

2,3-DIHYDROXYSUCCINONITRILE-2,3-H² [Glyoxal-H₂² Bis(cyanohydrin)]



H. Erlenmeyer, O. Bitterlin and H. M. Weber, *Helv. Chim. Acta*, 22, 701 (1939).

A. Procedure

(a) *Glyoxal-H₂² Bis(phenylhydrazone)*. A 15-1. flask is filled with ozonized oxygen, such that the concentration of ozone is 0.0075 g. per l.,

and 250 ml. of acetylene- H_2^2 is added slowly, according to the procedure of Wohl and Bräunig.¹ The glyoxal- H_2^2 is precipitated with finely atomized water and water vapor. From the aqueous condensate, the glyoxal is obtained as the phenylhydrazone. Pure glyoxal- H_2^2 bis(phenylhydrazone), m.p. 170–171°, is obtained by recrystallization of the crude product from alcohol (Note 1).

(b) 2,3-Dihydroxysuccinonitrile-2,3- H_2^2 , [Glyoxal- H_2^2 Bis(cyanohydrin)]. The cyanohydrin is prepared from the glyoxal- H_2^2 bisulfite addition product according to the procedure of Newman and Riley² (Note 2). A suspension of the bisulfite compound (55 g.) in 200 ml. of water is shaken with the equivalent amount of potassium cyanide, which is added slowly in the minimum amount of water.

B. Notes

1. Since the hydrogens of acetylene will exchange³ with hydrogen- H_2^2 in the presence of base or strong acid, exchange of hydrogen in this synthesis of glyoxal was a possibility. Exchange did not occur since the product was shown to contain 1.98 atoms of hydrogen- H^2 per molecule.

2. The cyanohydrin was not isolated by either Erlenmeyer, *et al.* or Newman and Riley.² In both instances, tartaric acid was prepared by acid hydrolysis of the cyanohydrin.

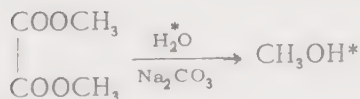
¹A. Wohl and K. Bräunig, Chem. Ztg., 44, 157 (1920); through Chem. Zentr., 1920, [4], 472.

²A. C. C. Newman and H. L. Riley, J. Chem. Soc., 1933, 45.

³L. H. Reyerson and B. Gillispie, J. Am. Chem. Soc., 58, 282 (1936).

METHANOL- H^2

METHOD I



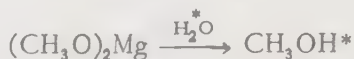
J. Beersmans and J. C. Jungers, Bull. soc. chim. Belges, 56, 72 (1947).

A. Procedure

In the reaction flask of an all-glass apparatus (Note 1) are placed 1 mole each of dry methyl oxalate, anhydrous sodium carbonate and water- H_2^2 . The mixture is heated under reflux for 12 hours (Note 2), and finally the methanol is all distilled into a cold receiver. The yield of methanol- H^2 is practically quantitative, but the product contains a small amount of

methanol oxalate which is removed by fractionation of the methanol-H² through a Podbielniak column. The product is 92% methanol-H².

METHOD II



F. W. Hobden, E. F. Johnston, L. H. P. Weldon and C. L. Wilson, J. Chem. Soc., 1939, 61.

A. Procedure

Magnesium is dissolved in absolute methyl alcohol, and the magnesium methoxide produced is dried at 100°, *in vacuo*. Less than 1 equivalent of water-H₂² (99.5%) is then added, and after the mixture is shaken for several hours, the methanol-H² is distilled under vacuum by warming the mixture to 60°. The product is 99.2% methanol-H².

B. Notes

1. The apparatus consists of a reaction flask surmounted by a reflux condenser, inclined at about 45°, which is attached to a receiver cooled with Dry Ice. The exit from the receiver is protected with a phosphorus pentoxide tube.

2. As the carbon dioxide is evolved some of the methanol-H² is entrained and is collected in the Dry Ice-cooled trap.

C. Other Preparations

Methanol-H² has been prepared from magnesium methoxide and water-H₂² in several instances¹⁻³ and from sodium methoxide and water-H₂².^{4,5}

¹E. Bartholomé and H. Sachsse, Z. physik. Chem., 30B, 40 (1935).

²O. Redlich and F. Pordes, Monatsh., 67, 203 (1936).

³J. O. Halford, L. C. Anderson and G. H. Kissin, J. Chem. Phys., 5, 927 (1937).

⁴D. G. Hill, B. Stewart, S. W. Kantor, W. A. Judge and C. R. Hauser, J. Am. Chem. Soc., 76, 5129 (1954).

⁵J. R. Johnson and V. J. Shiner, Jr., *ibid.*, 75, 1350 (1953).

METHANOL-H²-1-H₁²



J. Beersmans and J. C. Jungers, Bull. soc. chim. Belges, 56, 72 (1947).

A. Procedure

Methanol- H^2 -1- H_1^2 is prepared essentially according to the method of Halford¹ (Note 1). A stream of nitrogen is bubbled through a warm solution of diazomethane in ether. The mixture of diazomethane, ether vapor and nitrogen is passed upward through a water-cooled condenser and a second condenser at -20° (a Dry Ice-acetone type). The stream of nitrogen and diazomethane is then bubbled through a column of water- H_2^2 . The reaction is sufficiently slow that the rate is increased by moderate heating. The methanol- H^2 -1- H_1^2 is obtained by rectification of the aqueous solution through a Podbielniak column (Note 2).

B. Notes

1. Halford¹ catalyzed the reaction with sulfuric acid- H_2^2 .
2. The product contains some methanol- H^2 -1- H_2^2 .

C. Other Preparations

Methanol-1- H_1^2 has been prepared² by the reduction of formaldehyde- H_2^2 with sodium borohydride according to the method of Chaikin and Brown.³

Methanol- H^2 -1- H_1^2 has been prepared⁴ similarly to the method described, using phosphoric acid- H_3^2 as catalyst.

¹J. O. Halford, L. C. Anderson and G. H. Kissin, *J. Chem. Phys.*, **5**, 927 (1937).

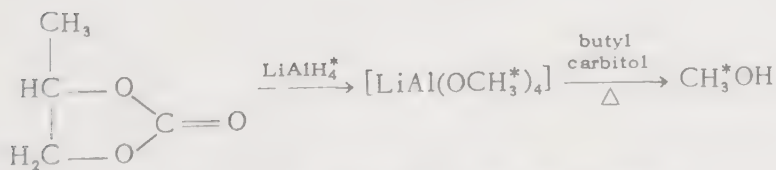
²J. R. Rachele, E. J. Kuchinskas, J. E. Knoll and M. L. Eidinoff, *J. Am. Chem. Soc.*, **76**, 4342 (1954).

³S. W. Chaikin and W. G. Brown, *ibid.*, **71**, 122 (1949).

⁴A. Langseth and B. Bak, *Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd.*, **24**, No. 3 (1947).

METHANOL-1- H_3^2

METHOD I



W. F. Edgell and L. Parts, *J. Am. Chem. Soc.*, **77**, 5515 (1955).

A. Procedure

To a stirred mixture of 17.3 g. (0.412 mole) of lithium aluminum hydride- H_4^2 and 200 g. of diethyl carbitol, under a nitrogen atmosphere, is

B. Notes

1. Other organic carbonates, e.g., ethylene carbonate, could probably be used.

2. Some hydrogen is evolved during the beginning of the butyl carbitol addition since 10% excess lithium aluminum hydride- H_4^2 was used. In the reduction of phosgene and carbon dioxide with lithium aluminum hydride, the yields of methanol, based on the hydride, are quite low since large excesses of lithium aluminum hydride must be used.

3. The all-glass apparatus, which is shown diagrammatically by Beersmans and Jungers, is the vacuum manifold type. The apparatus includes a cell for the electrolytic preparation of hydrogen- H_2^2 from water- H_2^2 and storage flasks for carbon monoxide, which is prepared by the dehydration of formic acid with sulfuric acid. The carbon monoxide is absorbed on activated carbon at -80° and then allowed to slowly desorb into the reaction chamber by the slow vaporization of a Dry Ice-bath.

4. The catalyst is prepared according to the procedure of Fenske and Frolich¹ from the nitrates of copper and zinc and chromic acid anhydride.

5. Runs producing up to 40 ml. of methanol were made.

6. According to Raman and infrared spectral analyses, the methanol- H_4^2 contained 98% deuterium in the methyl group and 97% in the hydroxyl group.

C. Other Preparations

Methanol- H_4^2 has been prepared² by the hydrolysis of methyl- H_3^2 bromide with a solution of sodium hydride- H^2 in water- H_2^2 . Methanol-1- H_3^2 has been prepared from methanol- H_4^2 by exchange.

Methanol- H_4^2 has also been prepared,³ in 80% yield, using a modification of the procedure described by Beersmans and Jungers (Method II); and from methanol-1- H_3^2 by exchange with water- H_2^2 at 200° in a sealed tube.⁴

Methanol- C^{14} -1- H_3^2 has been prepared⁵ by the reduction of carbon- C^{14} dioxide with lithium aluminum hydride- H_4^2 according to procedures^{6,7} described earlier.

¹M. R. Fenske and P. K. Frolich, *Ind. Eng. Chem.*, **21**, 1052 (1929).

²H. D. Noether, *J. Chem. Phys.*, **10**, 693 (1942).

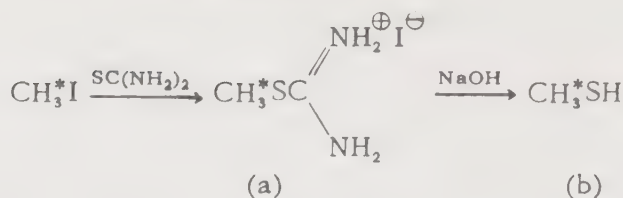
³M. Corval and R. Viallard, *Bull. soc. chim. France*, **21**, 484 (1954); *J. chim. phys.*, **51**, 619 (1954); M. Corval and C. Piolet, *ibid.*, **21**, 234 (1954).

⁴P. Venkateswarlu, H. D. Edwards and W. Gordy, *J. Chem. Phys.*, **23**, 1195 (1955).

⁵J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, *J. Biol. Chem.*, **215**, 593 (1955).

⁶R. F. Nystrom, W. H. Yanko and W. G. Brown, *J. Am. Chem. Soc.*, **70**, 441 (1948).

⁷J. D. Cox, H. S. Turner and R. J. Warne, *J. Chem. Soc.*, 1950, 3167.

METHANETHIOL-1-H₃²

N. Solimene and B. P. Dailey, J. Chem. Phys., 23, 124 (1955).

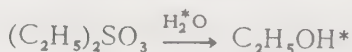
A. Procedure

(a) *S*-Methyl-H₃²-thiuronium Iodide. The *S*-alkylthiuronium salt is prepared by refluxing a mixture of iodomethane-H₃² and thiourea for several hours.

(b) *Methanethiol-1-H₃²*. The *S*-methyl-H₃²-thiuronium salt is hydrolyzed with sodium hydroxide solution. The resulting methanethiol-1-H₃² is purified by distillation under vacuum.

B. Other Preparations

Solimene and Dailey prepared methanethiol-H² by equilibrating methanethiol with water-H₂².

ETHANOL-H²

E. de Salas and C. L. Wilson, J. Chem. Soc., 1938, 319.

A. Procedure

Ethyl sulfite is hydrolyzed with slightly more than the equivalent amount of water-H₂². The ethanol-H² is dried over freshly ignited calcium oxide and then over magnesium amalgam.

B. Other Preparations

In a number of instances, ¹⁻³ ethanol-H² has been prepared by the hydrolysis of sodium ethoxide with water-H₂², and in others^{1,4} by the hydrolysis of aluminum ethoxide. The reaction of ethyl orthocarbonate with water-H₂² in the presence of sulfuric acid catalyst has also been used⁵ to prepare ethanol-H² and gives maximum deuterium utilization but suffers from unavailability of the starting material.

¹T. L. Chang, *Science*, 100, 30 (1944).

²P. S. Skell and C. R. Hauser, *J. Am. Chem. Soc.*, 67, 1661 (1945).

³S. Mizushima, Y. Morino and G. Okamoto, *Bull. Chem. Soc. Japan*, 11, 553 (1936).

⁴S. J. Cristol and D. D. Fix, *J. Am. Chem. Soc.*, 75, 2647 (1953).

⁵J. D. Roberts, C. M. Regan and I. Allen, *ibid.*, 74, 3679 (1952).

ETHANOL-1-H²

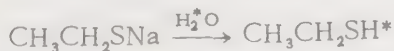


H. F. Fisher, E. E. Conn, B. Vennesland and F. H. Westheimer, *J. Biol. Chem.*, 202, 687 (1953); *J. Am. Chem. Soc.*, 73, 2403 (1951).

Procedure

In a three-necked flask equipped with a reflux condenser, a dropping funnel and a stirrer, a suspension of 7 g. (0.17 mole) of lithium aluminum hydride-H₄² in 500 ml. of ethyl ether is stirred under a nitrogen atmosphere for 2 hours. Then 0.3 mole of phenyl acetate in 40 ml. of ether is added at such a rate as to maintain gentle refluxing. After the reaction mixture is refluxed for an additional 2 hours, it is treated cautiously first with wet ether and then with 50 ml. of water. The resulting mixture is made alkaline to litmus, and an additional 0.1 mole of sodium hydroxide is added in order to saponify any ethyl acetate formed by ester exchange. Fractionation of the mixture through an 18-inch Podbielniak column gives the water-ethanol-1-H₂² azeotrope. The fraction boiling from 77.8–78.3° is dried over calcium oxide; the yield of ethanol-1-H₂² based on lithium aluminum hydride-H₄² is 55% (deuterium content, 2.06 atoms per molecule).

ETHANETHIOL-H²



F. W. Hobden, E. F. Johnston, L. H. P. Weldon and C. L. Wilson, *J. Chem. Soc.*, 1939, 61.

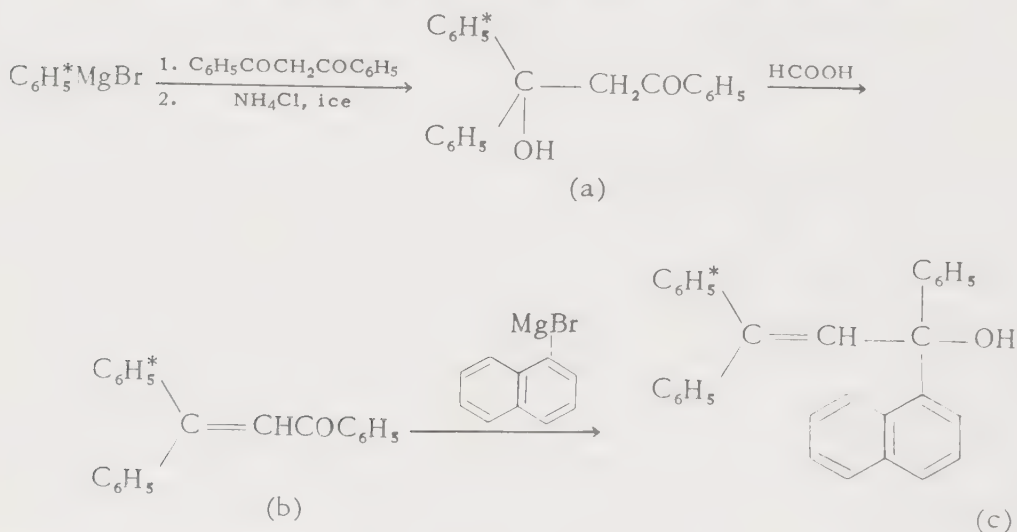
A. Procedure

Sodium metal, 3 g., is dissolved in 20 ml. of dry ethanethiol. The resultant sodium ethanethiolate is treated with excess water-H₂² and dry carbon dioxide. The ethanethiol-H₂² is distilled, under vacuum into a trap immersed in a Dry Ice-acetone bath. After drying over potassium carbonate, the product is distilled in vacuum.

B. Other Preparations

1-Butanethiol- H^2 has been prepared¹ by the treatment of sodium 1-butanethiolate with hydrochloric acid- H^2 in water- H_2^2 . The yield was practically quantitative, but it was necessary to use a large excess of the acid solution.

¹L. A. Wall and D. W. Brown, J. Polymer Sci., 14, 513 (1954).

1-(1-NAPHTHYL)-1,3-DIPHENYL-3-PHENYL- H_5^2 -2-PROPEN-1-OL

G. R. Clemo, R. Roper and A. C. Robson, J. Chem. Soc., 1939, 431.

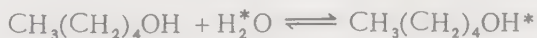
A. Procedure

(a) *3-Hydroxy-3-phenyl-3-phenyl- H_5^2 -propiophenone*. 1,3-Diphenyl-1,3-propanedione, 0.4 g., in 1 ml. of benzene is added, during 15 minutes, to phenylmagnesium- H_5^2 bromide (from 0.2 g. of bromobenzene- H_5^2) at 10° . The bright green solution becomes yellow overnight. After the magnesium compound is hydrolyzed with ice and ammonium chloride, the ether layer is dried and evaporated. The residual gum is dissolved in a minimum of benzene, and about 4 ml. of petroleum ether is added. The solid obtained is recrystallized from alcohol; yield 0.3 g., m.p. 115° .

(b) *β -Phenyl- H_5^2 -chalcone*, (2-Benzylidene-2-phenyl- H_5^2 -acetophenone). The above propiophenone, 0.2 g., is dehydrated by heating under reflux for 30 minutes with 5 ml. of formic acid (d 1.2). The mixture is diluted with water; the product is extracted with benzene, which is dried over potassium carbonate and evaporated to dryness. After the residue is crystallized from a few ml. of alcohol, the yield of golden-yellow prisms is 0.18 g., m.p. $85-86^\circ$.

(c) 1-(1-Naphthyl)-1,3-diphenyl-3-phenyl- H_5^2 -2-propen-1-ol. β -Phenyl- H_5^2 -chalcone, 0.12 g., in 1 ml. of benzene is added during 5 minutes to the Grignard reagent prepared from 0.25 ml. of 1-bromonaphthalene. After 12 hours, the reaction mixture is hydrolyzed with ice and ammonium chloride, the organic layer is dried, and the solvent is removed. The residue is dissolved in a minimum of methyl alcohol, and the product, 0.1 g. (m.p. 114–115°), is slowly deposited; after recrystallization from ethyl acetate-alcohol, m.p. 117–119°.

1-PENTANOL- H^2



F. W. Hobden, E. F. Johnston, L. P. H. Weldon and C. L. Wilson, J. Chem. Soc., 1939, 61.

A. Procedure

Complete replacement of the hydroxyl hydrogen in amyl alcohol is effected by successive equilibrations with pure water- H_2^2 . The reactants are shaken together (Note 1) and cooled to -10° (Note 2), and the upper alcoholic layer is transferred, without contact with the atmosphere, into a second bulb containing fresh water- H_2^2 . Treatment with four successive portions of water- H_2^2 (99.6%) is followed by equilibration with two successive portions of water- H_2^2 (99.95%). The alcohol is dried over ignited potassium carbonate and finally over aluminum amalgam. The 1-pentanol- H^2 is distilled *in vacuo* (Note 3).

B. Notes

1. Equilibrium is attained in approximately 3 hours at 25°, $k = 0.57$, and the distribution coefficient $\left(\alpha = \frac{H^2}{H} \text{ alcohol} \div \frac{H^2}{H} \text{ water} \right)$ is 1.09 at 25°.

2. In order to diminish mutual solubilities and also to freeze the water layer.

3. Vapor pressure data at various temperatures for 1-pentanol- H^2 and 1-pentanol are given by Hobden. The ratio of vapor pressures for the heavy and normal alcohols at 25° is 0.855. According to the deuterium analysis, the product was 99.9% $C_5H_{11}OH^2$.

C. Other Preparations

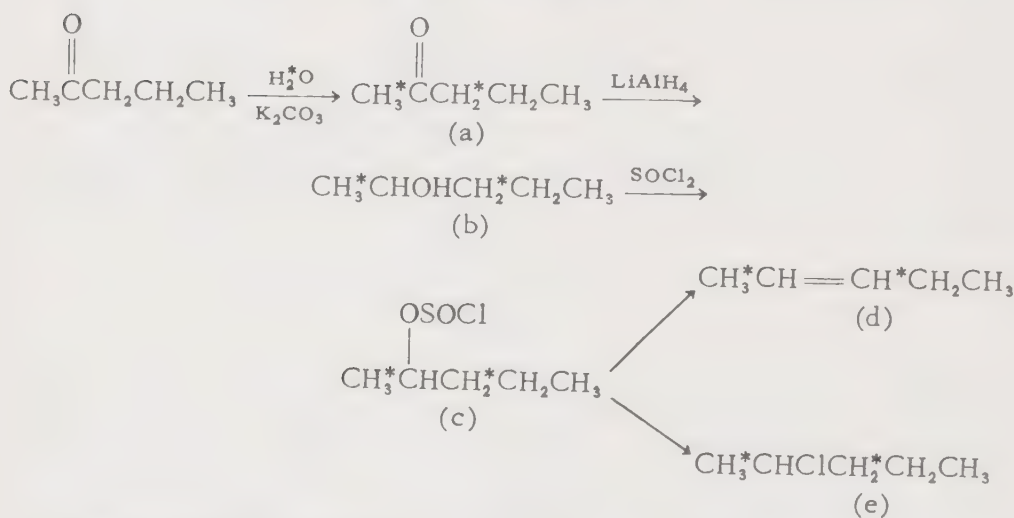
A quantity of 1-pentanol- H^2 , containing about 66 mole % of $C_5H_{11}OH^2$, has been prepared¹ by shaking equimolar amounts of amyl alcohol and water- H_2^2 (99%) for 10 minutes.

1-Pentanol- H^2 , 1-butanol- H^2 , 2-methyl-1-propanol- H^2 and 1-propanol- H^2 have all been prepared² in a dry box by treatment of the corresponding magnesium alkoxide with water- H^2 .

¹F. A. Hochstein and W. G. Brown, J. Am. Chem. Soc., 70, 3484 (1948).

²J. R. Quinan and S. E. Wiberley, Anal. Chem., 26, 1762 (1954).

2-PENTANOL-1,3- H^2



C. E. Boozer and E. S. Lewis, J. Am. Chem. Soc., 76, 794 (1954).

A. Procedure

(a) *2-Pentanone-1,3- H^2* . Five samples of 2-pentanone, 22 g. in each, are placed in 5 flasks equipped with reflux condensers. A 10-g. sample of water- H^2 , containing 0.1 g. of potassium carbonate, is added to the first ketone sample, and the mixture is refluxed for 16 hours. The mixture is cooled, and the aqueous phase is transferred to the next sample of ketone with a pipet. This process is repeated until the water- H^2 has been exchanged, in turn, with all 5 samples of ketone. After the 5 exchanges the last sample of ketone is recovered by distillation and returned to its flask. This process is repeated 5 times with fresh samples of water- H^2 . Finally, 4 more samples of fresh water- H^2 are used, but each sample is introduced one flask farther down the line. The ketone samples are combined, excess water is removed by distillation, and the ketone is dried over Drierite. Distillation of the product yields 100 g. of 2-pentanone-1,3- H^2 , b.p. 101.9–102.0° (uncor.).

(b) *2-Pentanol-1,3- H^2* . The above 2-pentanone-1,3- H^2 is reduced with a slight excess of lithium aluminum hydride in ether solution. The yield of 2-pentanol-1,3- H^2 , b.p. 118.0–118.5° (uncor.), is 95 g. (Note 1).

(c) *1-Methyl- H^3 -butyl-2- H^2 Chlorosulfite*. The chlorosulfite is prepared¹ by adding a solution of 2-pentanol-1,3- H^2 in petroleum ether to a stirred

solution of thionyl chloride in petroleum ether at -20° , during a period of 2 hours. At the end of this addition most of the resulting hydrogen chloride is removed under vacuum. After the solution is left at room temperature for several hours, the petroleum ether and excess thionyl chloride are removed at room temperature with water-pump vacuum. The residue is vacuum-distilled (Notes 2 and 3).

(d) *2-Pentene-1,3- H_4^2* . According to the procedure described earlier,¹ about 10–25 g. of the chlorosulfite dissolved in dioxane is placed in a flask fitted with a gas inlet tube, a thermometer and a small reflux condenser. Dry nitrogen is bubbled through the solution and out through the top of the condenser into a trap containing water. After sufficient time for completion of the reaction (Note 4), the solution is fractionally distilled through a 3-foot column packed with glass helices. The fractions not consisting largely of solvent are refractionated to obtain fairly pure 2-pentene-1,3- H_4^2 , b.p. 36.0 – 36.5° (Note 5), in yields corresponding roughly to the yield of hydrogen chloride.

(e) *2-Chloropentane-1,3- H_5^2* . Since the boiling point of 2-chloropentane is only 4° below that of dioxane, the latter was removed from the product by washing the distillate with water. Yields of 2-chloropentane-1,3- H_5^2 and some kinetic data are given in the following table.

TABLE XVI, 5
Decomposition of 1-Methylbutyl Chlorosulfite

Chlorosulfite	Solvent	Temp., $^{\circ}$ C.	Alkyl chloride, %	Alkene, %	$k \times 10^4$, sec^{-1}
1-Methylbutyl	dioxane	61.5	51.0 ± 0.5	48.8 ± 0.8	2.18 ± 0.02
1-Methyl- H_3^2 - butyl-2- H_2^2	"	"	51.2 ± 0.3	1.48 ± 0.05
1-Methylbutyl	"	77.5	44.3 ± 0.4	9.3 ± 0.2
1-Methyl- H_3^2 - butyl-2- H_2^2	"	"	46.6	6.6 ± 0.2
1-Methylbutyl	isooctane	95.5	0.167 ± 0.002
1-Methyl- H_3^2 - butyl-2- H_2^2	"	"	0.050 ± 0.001

B. Notes

1. Lithium aluminum hydride reductions are discussed by W. G. Brown.²
2. This procedure differs only slightly from that of Gerrard.³
3. The boiling point¹ of 1-methylbutyl chlorosulfite was 37 – 38° (2.4 mm.). This chlorosulfite, as well as the *sec*-butyl and 1-methylheptyl compounds, decomposed fairly rapidly in the pure state but could be kept practically unchanged for a week or two in solution in dioxane or isooctane.

4. The extent of the reaction can be estimated by titration of the hydrogen chloride and sulfur dioxide collected in the water-trap.

5. Deuterium analyses indicated that of the hydrogen atoms in the 1 and 3 positions of the 2-pentanone, 2-pentanol and 2-pentene, respectively, 87, 86 and 85% were replaced by hydrogen- H^2 . The data in the table show that substitution of hydrogen- H^2 for hydrogen resulted in a significant retardation of the over-all rate, but the nearly unaltered yields of the alkyl chloride indicate that the rate of formation of alkyl chloride must be retarded just as much as that of the olefin. It was concluded that the elimination and substitution reactions both go by way of a solvated carbonium ion intermediate and that rate retardation by deuterium results from hyperconjugation in the transition state.⁴ In isoöctane the rate retardation is much greater, which is consistent with the view⁵ that this solvent can in no way participate in the ionization; thus the electron deficiency is higher and the hyperconjugation more important than it is in the case of the dioxane solvent, where the nucleophilic nature of the solvent assists the ionization and reduces the electron deficiency.

¹E. S. Lewis and C. E. Boozer, J. Am. Chem. Soc., 74, 308 (1952).

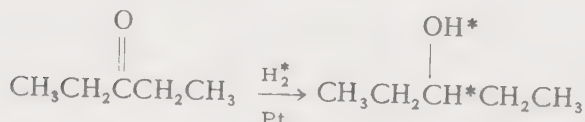
²*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 469.

³W. Gerrard, J. Chem. Soc., 1940, 218.

⁴E. S. Lewis and C. E. Boozer, J. Am. Chem. Soc., 76, 791 (1954).

⁵C. E. Boozer and E. S. Lewis, *ibid.*, 75, 3182 (1953).

3-PENTANOL- H^2 -3- H^2



L. C. Anderson and N. W. MacNaughton, J. Am. Chem. Soc., 64, 1456 (1942).

A. Procedure

3-Pentanone, 30 g., is hydrogenated with hydrogen- H^2 at 2 atmospheres pressure in the presence of 0.1-0.2 g. of platinum catalyst¹ promoted by iron. No additional solvent is necessary, and the reaction is carried out at 25°. When the reduction is complete, the product is frozen, and excess hydrogen- H^2 is pumped into a reservoir. The product is then distilled (Note 1).

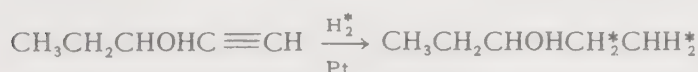
B. Notes

1. The hydrogen used in this study of the mechanism of hydrogenation with various catalysts was 20 atom per cent H^2 . The Raman spectra of the 3-pentanol- H^2 -3- H^2 and its oxidation products indicated direct addi-

tion of hydrogen to the carbonyl group, as compared to enolization followed by addition of hydrogen to an ethylenic bond. Apparently there was no exchange of hydrogen- H^2 for hydrogens on the carbons adjacent to the carbonyl.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

3-PENTANOL-1,2- H_2^2



F. C. McGrew and R. Adams, *J. Am. Chem. Soc.*, 59, 1497 (1937).

A. Procedure

In a hydrogenation apparatus¹ with a reduction vessel of 125-ml. capacity, a solution of 4.7 ml. of L-1-pentyn-3-ol (Note 1) in 15 ml. of ethyl acetate is shaken with 1 g. of platinum oxide catalyst and hydrogen- H_2^2 (~100%). The solution is shaken for a total of 80 hours (Note 2), and a total of 1900 ml. (STP) of hydrogen- H_2^2 is absorbed. The product is fractionally distilled; boiling range 111.8-112.4° (740 mm.), d_4^{20} 0.8533, n_D^{20} 1.4081, MR_D 26.62.

B. Notes

1. McGrew and Adams give the preparation of DL-1-pentyn-3-ol, b.p. 121-124° (750 mm.); its 3,5-dinitrobenzoate ester, m.p. 91°; its hydrogen phthalate ester, m.p. 72°, and resolution of the latter.

2. The solution, slightly optically active after 20 hours, was shaken an additional 60 hours and then displayed no detectable rotation.

¹A. McLean and R. Adams, *J. Am. Chem. Soc.*, 58, 804 (1936).

3-PENTANOL-1,2,4,5- H_{10}^2



L. C. Leitch and A. T. Morse, *Can J. Chem.*, 31, 785 (1953).

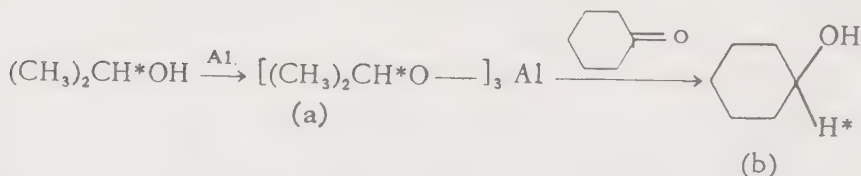
Procedure

The procedure of Lewis¹ is adapted to the preparation of 3-pentanol-1,2,4,5- H_{10}^2 . A solution of ethylmagnesium- H_{10}^2 bromide is prepared from 7.0 g. (0.287 mole) of magnesium turnings, 31.0 g. (0.272 mole) of bromoethane- H_2^2 and 100 ml. of absolute ether. The solution is cooled to -15°

and treated with 10.5 g. (0.142 mole) of ethyl formate over a period of 10 minutes. After the mixture is stirred for 15 minutes more, 30 ml. of water is added slowly, followed by a cold solution of 12 ml. of concentrated sulfuric acid in 60 ml. of water. The lower aqueous layer is separated and extracted with ether, and the extract is added to the main portion. The residue from the ether is fractionated, giving 10.6 g. (79.5%) of 3-pentanol-1,2,4,5- H^2_{10} , b.p. 113–114.5°, n_D^{20} 1.4050.

¹H. F. Lewis, J. Chem. Educ., 7, 856 (1930).

CYCLOHEXANOL-1- H^2



E. D. Williams, K. A. Krieger and A. R. Day, J. Am. Chem. Soc., 75, 2404 (1953).

A. Procedure

(a) *Aluminum 2-Propoxide-2- H^2* . 2-Propanol-2- H^2 , 100 ml. (1.31 moles), is heated under reflux with 13.5 g. (0.50 mole) of aluminum foil and 5 g. of aluminum isopropoxide added as catalyst. After 12 hours, the excess alcohol is distilled under vacuum, and 85.7 g. (83.3%) of aluminum 2-propoxide-2- H^2 is isolated.

(b) *Cyclohexanol-1- H^2* . Cyclohexanone (0.15 mole) is heated with a solution of 0.21 mole of aluminum 2-propoxide-2- H^2 in 150 ml. of carbon tetrachloride for 9.5 hours, under reflux (Note 1). After the acetone formed in the reaction and the carbon tetrachloride are removed under reduced pressure, hydrolysis is effected by heating the residue under reflux with 3.3 N sulfuric acid. The hydrolysis products are extracted into ether, dried and fractionated (Note 2). After a small amount of carbon tetrachloride-isopropyl alcohol azeotrope, isopropyl alcohol (b.p. 82–83°) is obtained and then cyclohexanol-1- H^2 , b.p. 33–35° (39–41 mm.) (Note 3).

B. Notes

1. In this study of the mechanism of the Meerwein-Ponndorf-Verley reaction, carbon tetrachloride was used as the solvent in the reduction. However, it was also demonstrated that higher yields (82–83%) of cyclohexanol are obtained with isopropyl or *t*-butyl alcohol as solvent. Since the amount of non-volatile polymer was least when the percentage re-

duction was highest, it was proposed that the tertiary alcohol inhibits the side reaction of aldolization.

2. A Todd column was used.

3. In a series of five experiments, the yields of cyclohexanol varied from 61.5 to 71.4%. The product is largely cyclohexanol-1- H^2 with about 7% of H^2 -cyclohexanol, which is formed by exchange. It was assumed that the amount of exchange is proportional to the initial methyl group deuterium content of the aluminum isopropoxide, which becomes available as H^2 -acetone is formed in the reaction. The experimental results indicate that reduction proceeds with hydrogen transfer mainly from the carbinol carbon atom of the isopropoxide group directly to the carbonyl carbon atom of the ketone. This is consistent with the suggested mechanism^{1,2} which involves a reaction complex with a cyclic structure for the essential hydrogen transfer step. Recent work^{3,4} on the stereochemical nature of the reaction also strongly supports the suggested mechanism.

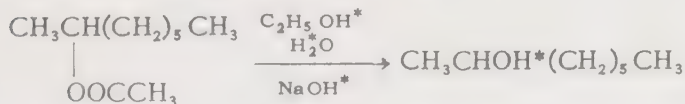
¹R. B. Woodward, N. L. Wendler and F. J. Brutschy, J. Am. Chem. Soc., 67, 1425 (1945).

²R. H. Baker and L. E. Linn, *ibid.*, 71, 1399 (1949).

³L. M. Jackson, A. Macbeth and J. Mills, J. Chem. Soc., 1949, 1425.

⁴W. E. Doering and R. W. Young, J. Am. Chem. Soc., 72, 631 (1950).

2-OCTANOL- H^2

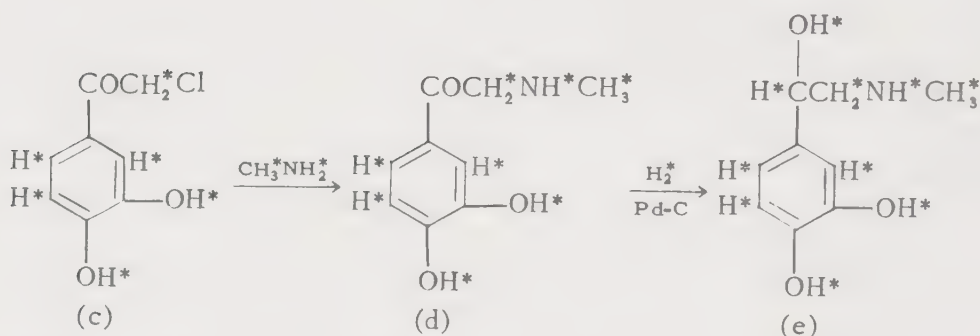
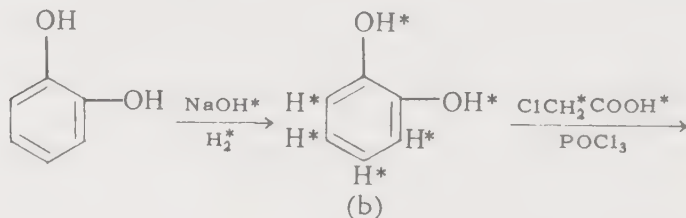
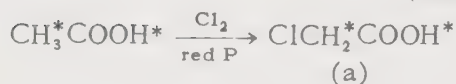


L. Young and C. W. Porter, J. Am. Chem. Soc., 59, 328 (1937).

Procedure

D-1-Methylheptyl acetate, 17.68 g., is dissolved in 120 ml. of ethanol- H^2 and 40 ml. of water- H_2^2 , and to this solution is added 10 g. of sodium hydroxide- H^2 . The mixture is heated under reflux for 1 hour on a water-bath. When most of the ethanol- H^2 is removed by distillation, the remaining mixture separates into two layers. The upper 2-octanol- H^2 layer is removed and dried with potassium carbonate; it is then fractionated to remove traces of ethanol and again dried. The process of alternate distillation and drying is repeated until there is no further change in rotatory power.

3,4-DIHYDROXY- α -(METHYLAMINOMETHYL)BENZYL- H^2_{12}
ALCOHOL- H^2
(Adrenaline- H^2_{13})



G. R. Clemo and G. A. Swan, J. Chem. Soc., 1942, 395.

A. Procedure

(a) *Chloroacetic- H^2_2 Acid- H^2* . Acetic- H^2_3 acid- H^2 is treated with chlorine in the presence of an iodine-red phosphorus catalyst, while heating on a water-bath. On fractionation, the distillate of b.p. $180\text{--}190^\circ$ crystallizes upon cooling. To remove remaining iodine, the product is placed over potassium hydroxide in a vacuum.

(b) *Catechol- H^2_6* . Catechol, 0.5 g., is placed in a bulb-tube with 1 g. of water- H^2_2 and 5 mg. of sodium hydroxide- H^2 . The tube is cooled, evacuated and sealed. After the reaction mixture is heated for 7 days at 200° , the tube is cooled and opened, and the water is distilled *in vacuo*. With a fresh 1 g. portion of water- H^2_2 added each time, the above process is repeated 3 times. Finally the catechol- H^2_6 is purified by sublimation (Note 1).

(c) *2-Chloro-3',4'-dihydroxyacetophenone- H^2_7* . A mixture of 0.12 g. of catechol- H^2_6 , 0.11 g. of chloroacetic- H^2_2 acid- H^2 and 0.08 g. of purified phosphoryl chloride (Note 2) is heated for 8 hours at $55\text{--}60^\circ$. Hot water- H^2_2 , 1.3 g., is then added and after 10–12 hours in the refrigerator, the 2-chloro-3',4'-dihydroxyacetophenone- H^2_7 is collected, washed with water- H^2_2 and dried. The crude product, 0.06 g., is recrystallized from water- H^2_2 , m.p. 172° .

(d) 3', 4'-Dihydroxy-2-methylaminoacetophenone- H_{11}^2 , (Adrenalone- H_{11}^2). 2-Chloro-3', 4'-dihydroxyacetophenone- H_7^2 , 0.12 g., is mixed with 0.35 g. of a 24% solution of methylamine- H_5^2 in water- H_2^2 . After 2 days at room temperature, the crude product (0.07 g.) is collected and washed with water- H_2^2 until the washings are no longer colored. Then, the crude product is dissolved in a small volume of hot dilute sulfuric acid- H_2^2 in water- H_2^2 , filtered while hot and cooled. The adrenalone- H_{11}^2 sulfate- H_2^2 which crystallizes is collected, washed with a little ethanol- H^2 and dried. The mother liquor yields an additional amount of the sulfate upon dilution with ethanol- H^2 and anhydrous ether.

(e) 3, 4-Dihydroxy- α -(methylaminomethyl)benzyl- H_{12}^2 Alcohol- H^2 , (Adrenaline- H_{13}^2). Adrenalone- H_{11}^2 sulfate- H_2^2 , 16 mg., is dissolved in 1.1 g. of water- H_2^2 , 3 mg. of palladium charcoal catalyst (Note 3) is added, and the mixture is shaken in an atmosphere of hydrogen- H_2^2 for 15 hours (Note 4). The catalyst is collected and washed with water- H_2^2 . To the combined filtrate is added a slight excess of anhydrous sodium carbonate (Note 5); the product soon begins to separate. After 2 hours (Note 6), the adrenaline- H_{13}^2 is collected, washed with water- H_2^2 and dried. The yield of white powder, m.p. 208° , is 10 mg.

B. Notes

1. By this procedure catechol- H_6^2 containing 93 atom per cent deuterium was obtained. In a similar procedure, the exchange mixture was heated for 3 periods of 4 days each at 100° . The residual catechol, extracted with ether, dried over sodium sulfate and recrystallized from dry light-petroleum (b.p. $80-100^\circ$), melted at 104° and contained 82 atom per cent deuterium.

2. The phosphoryl chloride was fractionally distilled from a little quinoline (to remove hydrogen chloride) out of contact with moisture.

3. The palladium-on-charcoal (10%) catalyst is heated for 1 hour at 80° and 15 mm. to remove most of the adsorbed moisture.

4. Hydrogen- H_2^2 was prepared by the action of water- H_2^2 on sodium metal in a vacuum apparatus. The hydrogen- H_2^2 was dried in passing through a trap cooled with liquid air and was collected in a mercury-filled reservoir with leveling bulb.

5. To free the base from the sulfate salt.

6. Out of contact with air.

2,3-BUTANEDIOL-2,3- H_2^2



A. Procedure

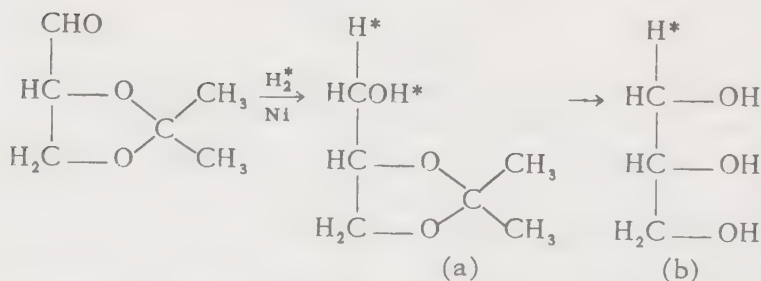
To a solution of 5 g. of lithium aluminum hydride- H_1^2 (Note 1) in 150 ml. of dry ether at -35° is added a solution of 20 g. of 2,3-butanedione in 100 ml. of ether (Note 2). After the reaction mixture is treated with aqueous mineral acid, the solution is extracted continuously with ether for 36 hours. The ether extract is dried, the ether is removed, and the product is distilled through a small all-glass column; b.p. $95-105^\circ$ (40 mm.). The 10 g. of distillate is presumably a mixture of the *meso* and racemic stereoisomers of 2,3-butanediol-2,3- H_2^2 .

B. Notes

1. Obtained from Metal Hydrides Inc., isotopic purity 99 + %.
2. For general information on lithium aluminum hydride reductions, see Brown.¹

¹*Organic Reactions*, Vol. 6, Wiley, New York, 1951, p. 469.

1,2,3-PROPANETRIOL-1- H_1^2
(Glycerol-1- H_1^2)



H. Erlenmeyer, H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, 20, 1012 (1937).

A. Procedure

(a) *1,2-Isopropylidene-1,2,3-propanetriol- H^2 -3- H_1^2* . A solution of freshly distilled isopropylidene-D-glyceraldehyde,¹ 7.8 g., and 1 g. of water- H_2^2 in 80 ml. of ethyl acetate, together with 10 g. of nickel catalyst,² is shaken at room temperature for 2 days with hydrogen- H_2^2 at 12 atmospheres pressure. The solution, freed of catalyst, is dried over potassium carbonate and concentrated under a column packed with glass beads. Vacuum distillation of the residue gives 6.0 g. of product, b.p. $78.5-79.5^\circ$ (11 mm.).

(b) *1,2,3-Propanetriol-1- H_1^2* . A solution of 4.05 g. of 1,2-isopropylidene-1,2,3-propanetriol- H^2 -3- H_1^2 in 4 ml. of 10% acetic acid is warmed for 30 minutes on a boiling water-bath. The solution is concentrated under a pressure of 12 mm. at $40-50^\circ$, and the oily residue is heated under vacuum for 1 hour at 160° . Distillation of the water-free residue affords 2.55 g. (91%) of 1,2,3-propanetriol-1- H_1^2 , b.p. $165-166^\circ$ (12 mm.).

¹H. O. L. Fischer and E. Baer, *Helv. Chim. Acta*, 17, 622 (1934).

²H. Rupe, A. Ackermann and H. Takagi, *Helv. Chim. Acta*, 1, 453 (1918).

2-AMINOETHANOL-1-C₁¹³/₁-2-C₁¹⁴/₁-1-H₂²



A. Weissbach and D. B. Sprinson, *J. Biol. Chem.*, 203, 1031 (1953).

A. Procedure

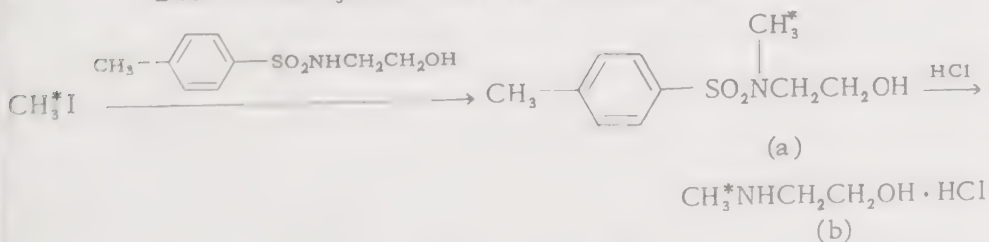
To a suspension of 600 mg. of glycine-1-C₁¹³/₁-2-C₁¹⁴/₁ ethyl ester hydrochloride in 0.8 ml. of chloroform, which is cooled in an ice-bath, is added dropwise, with stirring, 7 ml. of 2% ammonia in chloroform. After the cold mixture is stirred for an additional 15 minutes, it is centrifuged, and the precipitate is washed twice with cold chloroform. The combined supernatant solutions are evaporated to dryness *in vacuo*.

The residual glycine-1-C₁¹³/₁-2-C₁¹⁴/₁ ethyl ester is dissolved in a small amount of absolute ether and added dropwise to a solution of 420 mg. of lithium aluminum hydride-H₂² in 5 ml. of absolute ether, in a flask equipped with a magnetic stirrer, a reflux condenser and a dropping funnel. Fifteen minutes after the ester is added, 3 ml. of water-H₂² is added dropwise (Note 1). The resulting mixture is transferred to a continuous type extractor with 15 ml. of water and extracted for 48 hours with ether containing 3 ml. of 20% hydrochloric acid. The ether is evaporated, and the residue of crystalline amine hydrochloride is recrystallized from a small volume of hot absolute alcohol by the addition of dry ether. The yield of 2-aminoethanol-1-C₁¹³/₁-2-C₁¹⁴/₁-1-H₂², m.p. 74-76°, is 200 mg. (50%).

B. Notes

1. This water-H₂² was added to prevent a possible exchange of hydrogen between the lithium aluminum alkoxide and water. The necessity of such a precaution was considered quite doubtful.

2-METHYL-H₃^{*}-AMINOETHANOL HYDROCHLORIDE



V. du Vigneaud, J. P. Chandler, S. Simmonds, A. W. Moyer and M. Cohn, *J. Biol. Chem.*, 164, 603 (1946).

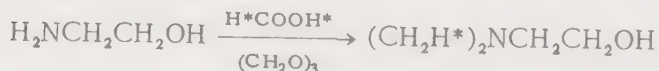
A. Procedure

(a) *N*-2-Hydroxyethyl-*N*-methyl- H_3^2 -*p*-toluenesulfonamide. Methyl- H_3^2 iodide (from 6.3 g. of methyl- H_3^2 alcohol) is distilled into a solution of 32 g. of *N*-2-hydroxyethyl-*p*-toluenesulfonamide¹ in 100 ml. of 3.5 *N* sodium hydroxide. This mixture is heated in a tightly stoppered flask at 65–75° for 90 minutes. The solution, after cooling to room temperature, is extracted several times with chloroform, and the combined extract is concentrated to dryness *in vacuo*. An alcoholic solution of the residue is decolorized with Darco, and the filtrate is evaporated to obtain 31 g. of product.

(b) 2-Methyl- H_3^2 -aminoethanol Hydrochloride. *N*-2-Hydroxyethyl-*N*-methyl- H_3^2 -*p*-toluenesulfonamide, 28 g., in 80 ml. of concentrated hydrochloric acid is heated in a sealed tube at 150° for 23 hours. When the acid solution is cooled to 0°, the *p*-toluenesulfonic acid crystallizes and is removed by filtration. The filtrate is concentrated to dryness *in vacuo*. The residue of 2-methyl- H_3^2 -aminoethanol hydrochloride is dissolved in absolute alcohol and precipitated by the addition of dry ether; yield 8.0 g.

(c) 2-Methyl- H_3^2 -aminoethanol Picrate. A sample of the amine hydrochloride is dissolved in concentrated potassium hydroxide solution, and the free amine is extracted with ether. The ether extract is dried over sodium sulfate and treated with an ether solution of picric acid. The picrate, which precipitates, is extracted with boiling ether to remove excess picric acid.

¹K. H. Slotta and R. Behnish, *J. prakt. Chem.*, 135, 225 (1932).

2-DIMETHYLAMINO-1,1'- H_2^2 -ETHANOL

V. du Vigneaud, J. P. Chandler, S. Simmonds, A. W. Moyer and M. Cohn, *J. Biol. Chem.*, 164, 603 (1946).

A. Procedure

2-Aminoethanol is methylated by the procedure of Clark.¹ To 40 ml. of solution containing 22 g. of formic- H^2 acid- H^2 (Note 1) is added 13 g. of trioxymethylene. To this solution, cooled in an ice-bath, is added 12 g. of 2-aminoethanol. The temperature is allowed to rise slowly (Note 2), and finally the mixture is refluxed for 4 hours. A slight excess of anhydrous hydrogen chloride is added to the solution, and volatile material is removed by distillation (Note 3). An aqueous solution of the residue is then made alkaline, and the mixture is distilled. The aqueous

distillate is saturated with potassium hydroxide, and the 2-dimethylamino-1,1'-H₂²-ethanol layer is removed, dried over anhydrous potassium carbonate and fractionally distilled (Note 4).

B. Notes

1. Introduction of deuterium in the preparation of 2-dimethylaminoethanol by this procedure was attempted with ordinary formic acid in a medium of water-H₂². The fact that the product contained only a trace of deuterium is in accord with the observation of Clark, *et al.*,¹ that the formic acid supplies the hydrogen required in the reduction.

2. This is to avoid a violent reaction.

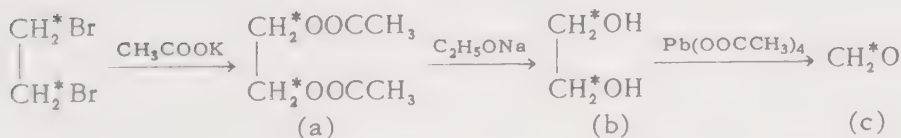
3. The exchangeable deuterium in the residue is removed by repeated equilibrations with ordinary water.

4. The product, after this treatment, was still wet and was indicated by titration to contain 84% of 2-dimethylamino-1,1'-H₂²-ethanol.

¹H. T. Clark, H. B. Gillespie and S. Z. Weiss Haus, J. Am. Chem. Soc., 55, 4571 (1953).

FORMALDEHYDE-H₂²

METHOD I



R. A. B. Bannard, A. T. Morse and L. C. Leitch, Can. J. Chem., 31, 351 (1953).

A. Procedure

(a) *Ethylene-H₄² Acetate* (Note 1). In a 500-ml. flask, fitted with a reflux condenser surmounted by a calcium chloride tube, are mixed 59 g. (0.602 mole) of fused potassium acetate, 51.0 g. (0.265 mole) of 1,2-dibromoethane-H₄² and 8 ml. of glacial acetic acid. The mixture is heated under reflux for 3 hours; then the liquid portion is removed by distillation, first at atmospheric pressure and finally at 20 mm. The distillate is fractionated to obtain 37.4 g. (94.0%) of product, b.p. 186-188°; n_D^{20} 1.4147; d_4^{20} 1.1372.

(b) *1,2-Ethanediol-1,2-H₄²* (Note 2). A sodium ethoxide solution is prepared from 0.5 g. of sodium and 150 ml. of absolute ethanol in a 500-ml. flask equipped with a reflux condenser and drying tube. To the sodium ethoxide is added 35.5 g. (0.236 mole) of ethylene-H₄² acetate in 100 ml. of absolute ethanol, and the resultant solution is refluxed for 6 hours.

The ethyl acetate and most of the ethanol are removed by distillation at atmospheric pressure. Fractionation of the residue, under vacuum, gives 13.8 g. (88.5%) of colorless product, b.p. $86-87^{\circ}$ (8 mm.); n_D^{20} 1.4293; d_4^{20} 1.1895.

(c) *Formaldehyde- H_2^2* . A vacuum manifold type of apparatus is used which is made up of three cold traps in series (Figure XVI, 2). The reaction flask, equipped with a magnetic stirrer and addition buret, is attached to the manifold through a vertical condenser. Lead tetraacetate, 56 g. (0.12 mole), is placed in the reaction flask and warmed gently for 30 minutes while the apparatus is evacuated to 0.05 mm. (Note 3). Dry air is admitted through a stopcock on the vacuum line, and 200 ml. of anhydrous benzene (Note 5) is added from the buret. Stirring is begun, water at 0° is circulated through the condenser, the first two traps are cooled in Dry Ice-acetone, and the mixture is gently refluxed. 1,2-Ethanediol- $1,2-H_4^2$, 6.2 g. (0.12 mole), is added dropwise from the buret, during 15 minutes. During the addition, the originally dark brown mixture becomes pale yellow, and a white precipitate of lead diacetate separates. Simultaneously, liquid formaldehyde- H_2^2 collects in the first trap and formaldehyde- H_2^2 polymer collects in the upper portion of the condenser and the tube leading to the condenser. The buret is rinsed with 10 ml. of anhydrous benzene, and the mixture is refluxed, with stirring, for another hour. The reaction vessel is cooled in an ice-bath, and the formaldehyde- H_2^2 polymer which has formed on the upper walls of the flask is depolymerized by gentle heating with a bare flame. The reaction flask

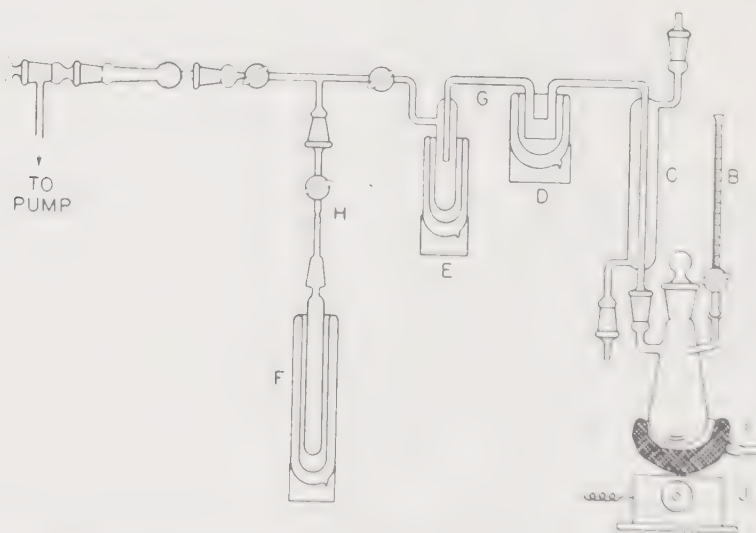


Fig. XVI, 2 Apparatus for the preparation of formaldehyde- H_2^2 (R. A. B. Bannard, A. T. Morse and L. C. Leitch). A, reaction flask; B, buret; C, condenser; D, E and F, cold traps; G and H, constrictions for sealing; I, heating mantle; J, magnetic stirring motor.

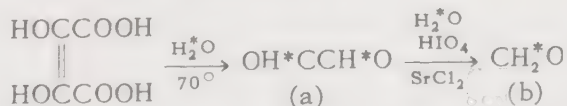
is disconnected, the lower end of the condenser is closed by means of a cap, and the second trap is immersed in liquid nitrogen. The system is evacuated to 0.1 mm., and the liquid formaldehyde- H_2^2 is distilled into the second trap as the first trap is allowed to warm slowly. After the distillation, the polymer in the condenser tube is depolymerized by passing hot nitrobenzene through the condenser jacket. At the same time the first trap and adjoining tubing are heated with a flame and then isolated by sealing off. The formaldehyde- H_2^2 is distilled *in vacuo* into the third receiver, which contains 20 ml. of anhydrous ether. The vessel is sealed and kept overnight in a Dry Ice-acetone bath at -78° , 12 hours at -25 to -10° and then for 3 days at 0° ; meanwhile, the polymer separates as a white flocculent solid (Note 6). After the vessel is cooled to -78° it is attached to the vacuum line, and the ether is distilled slowly from the polymer into a trap cooled with liquid nitrogen. Distillation of the ether is continued until the flask containing the formaldehyde- H_2^2 is at 35° . The formaldehyde- H_2^2 polymer weighs 4.8 g. (76%) (Note 7).

(d) *Ethylene-1,2- H_2^2 Acetate*. The procedure is the same as for the preparation of ethylene- H_4^2 acetate except that 50.0 g. (0.263 mole) of 1,2-dibromoethane-1,2- H_2^2 , 59.0 g. (0.602 mole) of fused potassium acetate and 8 ml. of glacial acetic acid are used. The yield of colorless product is 35.0 g. (90%), b.p. $185-188^\circ$; n_D^{20} 1.4150; d_4^{20} 1.1211.

(e) *1,2-Ethanediol-1,2- H_2^2* . In this case, 29.0 g. (0.196 mole) of ethylene-1,2- H_2^2 acetate, 250 ml. of absolute ethanol and 0.5 g. of sodium are used in the above procedure for 1,2-ethanediol-1,2- H_4^2 . 1,2-Ethanediol-1,2- H_2^2 is obtained as a colorless liquid, b.p. $86-87^\circ$ (8 mm.); n_D^{20} 1.4302; d_4^{20} 1.1506. The yield is 10.2 g. (81.6%).

(f) *Formaldehyde- H_2^2* . 1,2-Ethanediol-1,2- H_2^2 is oxidized in the same manner as 1,2-ethanediol-1,2- H_4^2 . The yield of formaldehyde- H_1^2 polymer is 4.65 g. (75%).

METHOD II



D. Elwyn, A. Weissbach, S. S. Henry and D. B. Sprinson, J. Biol. Chem., 213, 281 (1955).

A. Procedure

(a) *Glyoxal- H_2^2* . A solution of 51 g. (0.34 mole) of anhydrous dihydroxymaleic acid (Note 8) in 300 ml. of water- H_2^2 is heated at 70° until evolution of carbon dioxide ceases (Note 9).

(b) *Formaldehyde- H_2^2* . To the above solution, cooled to 10° , is added 68.4 g. (0.3 mole) of periodic acid in 50 ml. of water. After 20 minutes,

40 g. (0.15 mole) of strontium chloride is added, and the solution is adjusted to pH 6 with 1 *N* sodium hydroxide and refrigerated overnight. The solution is filtered to remove insoluble strontium salts. Then the filtrate is concentrated to 40 ml. by distillation and is steam-distilled until a total of 500 ml. of distillate is collected. The yield of formaldehyde- H_2^2 (Note 10) is 0.11 mole (33%).

B. Notes

1. This compound is prepared by a modification of the method of Henry.¹ The use of silver acetate² greatly reduces the yield.

2. H_4^2 -Glycol is prepared from the diacetate by a modification of the transesterification procedure of Bainbridge.³

3. There are a number of good syntheses of formaldehyde (see formaldehyde- C^{14}), but the product is obtained in dilute aqueous solution. According to Walker,⁴ the isolation of monomeric formaldehyde from aqueous solution by distillation is not realizable in practice, although theoretically possible. Under the best conditions of formaldehyde recovery, the end product is paraformaldehyde containing 7% combined water.⁵ The cleavage of ethylene glycol to two moles of formaldehyde can be nearly quantitative, but such reagents as periodic acid,^{6,7} sodium bismuthate,⁸⁻¹⁰ trivalent silver ion⁸ and chromyl chloride¹¹ must be used in aqueous solution. Hence, attention was centered on the use of lead tetraacetate in organic solvents.^{12,13}

4. Solutions of formaldehyde in nonhydroxylic solvents evolve monomeric formaldehyde gas almost quantitatively on warming to room temperature.¹⁴ Benzene was selected as solvent because it can be readily dried and dissolves more lead tetraacetate than most other nonhydroxylic solvents.¹³

5. The formaldehyde, dissolved in ether, polymerizes slowly to eupolyoxymethylene on standing at 0°. Explosions occur in the polymerization step if the ether solution warms too rapidly.

6. Glycol cleavage with lead tetraacetate proceeds only if the hydroxyl groups of the glycol are unsubstituted.¹³ It was logical to conclude from this observation that the two hydrogen atoms lost from the glycol during dialdehyde cleavage, which appear ultimately as acetic acid, are hydrogen atoms originally bound to oxygen rather than carbon. As proved to be the case, it was therefore considered unnecessary to prepare 1,2-ethanediol- H_2^2 as the requisite intermediate.

7. The acid was dried to constant weight at 78°, *in vacuo*, over phosphorus pentoxide.

8. It has been observed¹⁵ that dihydroxymaleic acid decarboxylates readily at 50° in ordinary water but not noticeably in water- H_2^2 at this temperature.

9. The yield was determined by bisulfite titration.

C. Other Preparations

Formaldehyde- H_2^2 has been prepared¹⁶ by irradiating a mixture of carbon monoxide and hydrogen- H_2^2 and by the decomposition of stannous formate- H_2^2 at 180° .¹⁷

For purposes of analysis, Bannard, Morse and Leitch¹⁷ prepared dichloromethane- H_1^2 and dichloromethane- H_2^2 by the reaction of formaldehyde- H_1^2 and formaldehyde- H_2^2 , respectively, with phosphorus pentachloride.

Dichloromethane- H_1^2 and - H_2^2 have also been prepared,¹⁸ respectively, by the reaction of chloroform, acetic acid- H^2 and zinc, and by the reaction of chloroform- H^2 , acetic acid- H^2 and zinc.

¹L. Henry and P. Henry, *Bull. acad. roy. Belg.*, (3) 32, 402 (1896).

²A. Wurtz, *Ann. chim. et phys.*, (3) 55, 406 (1859).

³E. G. Bainbridge, *J. Chem. Soc.*, 105, 2291 (1914).

⁴J. F. Walker, *Formaldehyde*, Reinhold Publishing Corporation, New York, 1944, p. 58.

⁵I. G. Farbenindustrie, A. G., German Patent 503,180 (1930).

⁶*Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 346.

⁷L. Malaprade, *Bull. soc. chim. France*, (4) 43, 683 (1928).

⁸L. J. Heidt, E. K. Gladding, C. B. Purves, *Paper Trade J.*, 121 (9), 81 (1945).

⁹J. D. Cox and R. J. Warne, *J. Chem. Soc.*, 1951, 1893.

¹⁰W. Rigby, *ibid.*, 1950, 1907.

¹¹R. Slack and W. A. Waters, *ibid.*, 1949, 594.

¹²R. Criegee, *Ber.*, 64, 260 (1931).

¹³R. Criegee, L. Draft and B. Rank, *Ann.*, 507, 171 (1933).

¹⁴Reference 1, p. 28.

¹⁵D. Elwyn, S. S. Henry and D. B. Sprinson, unpublished observations.

¹⁶E. S. Ebers and H. Nielsen, *J. Chem. Phys.*, 6, 311 (1938).

¹⁷R. A. B. Bannard, A. T. Morse and L. C. Leitch, *Can. J. Chem.* 31, 351 (1953).

¹⁸R. J. Meyers and W. D. Gwinn, *J. Chem. Phys.*, 20, 1420 (1950).

ACETALDEHYDE-1- H^2

METHOD I



L. C. Leitch, *Can. J. Chem.*, 33, 400 (1955).

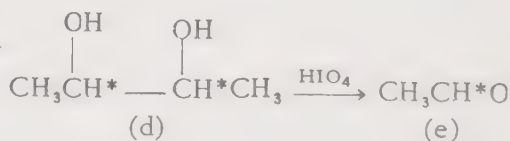
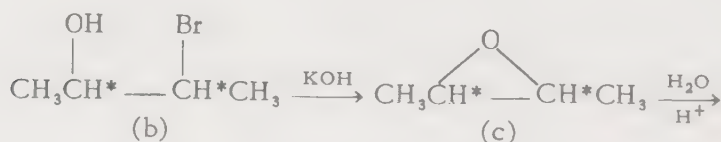
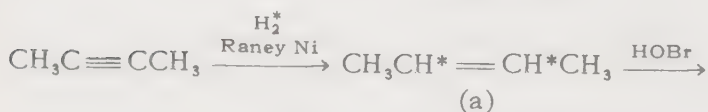
A. Procedure

(a) *Nitroethane-1- H_2^2* . To a mixture of 25 ml. of nitroethane and 25 ml. of water- H_2^2 is added 10 mg. of anhydrous sodium acetate. The tube containing the mixture is then sealed and heated in a rocking furnace overnight at 90° . The tube containing the deep-yellow reaction mixture is

opened, attached to a vacuum line, immersed in a freezing mixture, and evacuated. The upper layer of nitroethane-1- H_2^2 is distilled *in vacuo* through a U-tube containing Drierite and condensed in a U-tube at -78° . The yield of colorless product is 19 ml. (Note 1). This material is treated in the same manner with an equal volume of fresh water- H_2^2 containing sodium acetate. The final yield of nitroethane-1- H_2^2 is 11.0 ml. (Note 2).

(b) *Acetaldehyde-1- H^2* . Nitroethane-1- H_2^2 , 3.0 ml., is dissolved in 20 ml. of ice-cold 10% sodium hydroxide in a small separatory funnel. The solution is slowly added dropwise to a stirred solution of 6 ml. of sulfuric acid in 40 ml. of water, which is kept at 0 to 5° . The addition of the nitroethane-1- H_2^2 solution produces a characteristic blue color in the acid medium which fades as nitrous oxide is evolved. Acetaldehyde-1- H^2 which is entrained in the nitrous oxide is condensed in a U-tube cooled in a Dry Ice-acetone bath at -40° . After the addition of the basic solution is complete, stirring is continued for 15 minutes. Then the reaction mixture is heated, and the acetaldehyde-1- H^2 is distilled into the U-tube. The product is distilled from the U-tube into a graduated trap on the vacuum line; the yield is 0.8 ml. (Notes 3 and 4).

METHOD II



F. E. Blacet and R. K. Brinton, J. Am. Chem. Soc., 72, 4715 (1950).

A. Procedure

(a) *2-Butene-2,3- H_2^2* . The catalytic half-reduction of 2-butyne is carried out in a closed system by circulating hydrogen- H_2^2 , at about 2 atmospheres, through a solution of the butyne in ether. A solution of 10.9 g. of 2-butyne in 25 ml. of ether, containing a suspension of 0.5 g. of Raney nickel catalyst,¹ is treated with 5090 ml. of hydrogen- H_2^2 (99.8%) during a period of 4 hours. The gas stream is circulated through the ether solution at a rate of 200 ml. per minute. Solvent and dissolved hydrocarbons

carried out of the solution by the gas stream are condensed and returned by a cold-finger condenser at -80° .

(b) *threo*-3-Bromo-2-butanol-2,3- H_2^2 . The procedure used is a modification² of the procedure of Winstein and Lucas.³ In a 3-necked, 500-ml. flask, equipped with a mechanical stirrer and Dry Ice-alcohol reflux condenser, is mixed 0.4 mole of N-bromoacetamide, 250 ml. of water and 4 ml. of 6 N sulfuric acid. With the flask cooled in an ice-bath, 0.4 mole of 2-butene is added to the mixture. During 1.5 to 2 hours of stirring the mixture, the 2-butene disappears. The reaction mixture is extracted with three 100-ml. portions of ether. The extracts are combined, washed with sodium bicarbonate solution and dried over sodium sulfate. After removal of ether, the product is distilled at reduced pressure. The yield of *threo*-3-bromo-2-butanol-2,3- H_2^2 , b.p. $49.5-51^{\circ}$ (13 mm.), is 72.5-82%.

(c) *cis*-2,3-Epoxybutane-2,3- H_2^2 . The following procedure is a modification³ of that described by Wilson and Lucas.⁴ In a 100-ml. 3-necked flask, equipped with a mercury-sealed stirrer, a distillation condenser, a dropping funnel and a thermometer extending nearly to the bottom of the flask, 0.5 mole of potassium hydroxide is dissolved in 20 ml. of water. With the stirred solution at a temperature of 90° , 0.1 mole of *threo*-3-bromo-2-butanol-2,3- H_2^2 is added dropwise (Notes 5 and 6). The distillate is dried over anhydrous potassium carbonate, filtered and distilled; yield, 6.0 g. (83%) (Note 7).

(d) 2,3-Butanediol-2,3- H_2^2 . This diol is prepared according to the procedure of Wilson and Lucas.⁴ To a mixture of 300 ml. of water and 90 grams of 2,3-epoxybutane-2,3- H_2^2 is added 6 drops of 60% perchloric acid. The tightly stoppered flask is agitated and cooled with tap water intermittently for 5-10 minutes, when the undissolved oxide goes into solution. The solution is neutralized at the end of an hour and then fractionally distilled at reduced pressure (Note 8); yield, 101-107 g. (90-95%). The 2,3-butanediol-2,3- H_2^2 is recrystallized from dry isopropyl ether by cooling the solution to temperatures near -80° . After 5 or 6 recrystallizations, the pure diol melts at 7.6° and boils at 86° (16 mm.).

(e) Acetaldehyde-1- H^2 . The aldehyde is prepared from 2,3-butanediol-2,3- H_2^2 , by periodate oxidation according to the procedure of Birkinshaw.⁵ A standard solution of potassium periodate is added in slight excess to an aqueous solution of the diol. The solution is acidified with 10% sulfuric acid and stored at room temperature for 12 hours. The diol is completely converted to acetaldehyde-1- H^2 , which is purified by preparation of the aldehyde ammonia compound and regeneration with sulfuric acid (Notes 9 and 10).

B. Notes

1. The loss of nitroethane was due to the formation of a water-soluble by-product which was not further investigated.

2. The mass spectrum of the product indicated about 45 mole per cent of nitroethane-1- H_2^2 and unknown amounts of nitroethane-1- H_1^2 and nitroethane.

3. The deuterated nitroethane was subjected to the Nef reaction⁶ essentially as described by Johnson and Degering.⁷ The mass spectrum of the acetaldehyde indicated that it was largely acetaldehyde-1- H^2 . This result indicates that the mechanism of the Nef reaction proposed by Mahler⁸ is incorrect but supports the simpler mechanism of van Tamelen and Thiede⁹. The latter interpretation of the Nef reaction does not account for the appearance of a transient blue color in the reaction mixture prior to the evolution of nitrous oxide. Nametkin¹⁰ attributed this color to the formation of a nitroso intermediate. Leitch has suggested a modification of the mechanism of van Tamelen and Thiede to include a nitroso intermediate.

4. The Nef reaction is well adapted to the synthesis of acetaldehyde-1- H^2 and other aldehydes deuterated only in the formyl group on account of its simplicity and the high isotopic purity of the product. In this case, the product was over 95% acetaldehyde-1- H^2 if the results are based on the theoretical calculation of Brinton and Blacet¹¹ for the mass ratio of $CH^2O:CHO$ obtained spectrometrically. The apparent discrepancy between the deuterium content of the nitroethane-1- H_2^2 and the acetaldehyde-1- H^2 prepared from it may be reconciled if the data of Wynne-Jones¹² are considered. He gives 10:1 as the relative rates of ionization of the hydrogen and deuterium atoms of nitromethane. Therefore, assuming that the labeled nitroethane contained 50% of the isotopic species, $CH_3CHH^2NO_2$, its ion, $CH_3CH^2 = NO_2^-$, would be present to the extent of 45%; this amount, added to the 45% from $CH_3CH_2^2NO_2$, would lead to acetaldehyde containing 90% CH_3CH^2O .

5. The receiver is cooled in an ice-bath.

6. A slow current of air drawn through the apparatus, at the end of the reaction, will carry over some additional oxide.

7. On distillation through a Weston column, 80% of the distillate was collected at 59.8–60.1° and 20% at 58.2–59.8°, indicating that the product was essentially pure *cis*-epoxide.

8. The pressure is gradually reduced so that the temperature does not exceed 100°.

9. The mass spectrometric analysis¹³ of this acetaldehyde-1- H^2 indicated the following composition: CH_3CHO , 17.1%; CH_3CH^2O , 78.2%; CH_2H^2CHO , 1.8%; $CH_2H^2CH^2O$, 1.4%; CHH^2CHO , 0.4%; CHH^2CH^2O , 0.7%; CH_3^2CHO , trace; $CH_3^2CH^2O$, 0.4%.

10. For general information regarding the periodate oxidation method see *Organic Reactions*.¹⁴

C. Other Preparations

Acetaldehyde-1-H² has been prepared by chromic acid oxidation of ethanol-1-H²¹⁵ and isolated as acetaldehyde-1-H² *p*-nitrophenylhydrazone. A considerable enrichment of deuterium occurred, from 40 to 60 atom per cent. It was established that this was not due to intramolecular shift of deuterium but rather to a preferential destruction of nonisotopic acetaldehyde by the chromic acid. Acetaldehyde-1-H² has also been obtained¹⁶ by the periodic acid oxidation of 2,3-butanediol-2,3-H² obtained from 2,3-butanedione by lithium aluminum hydride-H₄ reduction.

¹L. W. Covert and H. Adkins, *J. Am. Chem. Soc.*, **54**, 4116 (1932).

²S. Winstein and R. E. Buckles, *ibid.*, **64**, 2780 (1942).

³S. Winstein and H. J. Lucas, *ibid.*, **61**, 1576 (1939).

⁴C. E. Wilson and H. J. Lucas, *ibid.*, **58**, 2396 (1936).

⁵J. H. Birkinshaw, J. H. V. Charles and P. W. Clutterbuck, *Biochem. J.*, **25**, 1527 (1931).

⁶J. U. Nef, *Ann.*, **280**, 263 (1894).

⁷K. Johnson and E. F. Degering, *J. Org. Chem.*, **8**, 10 (1943).

⁸H. R. Mahler, U. S. A. E. C. Document No. 2400, 1948, p. 40.

⁹E. E. van Tamelen and R. J. Thiede, *J. Am. Chem. Soc.*, **74**, 2615 (1954).

¹⁰S. S. Nametkin, *J. Russ. Phys. Chem. Soc.*, **45**, 1414 (1913); *Chem. Abstracts*, **8**, 324 (1914).

¹¹F. E. Blacet and R. K. Brinton, *J. Am. Chem. Soc.*, **22**, 4715 (1950).

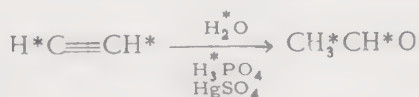
¹²W. F. K. Wynne-Jones, *J. Chem. Phys.*, **2**, 381 (1934).

¹³R. K. Brinton and F. E. Blacet, *J. Chem. Phys.*, **17**, 797 (1949).

¹⁴*Organic Reactions*, Vol. II, Wiley, New York, 1944, p. 341.

¹⁵J. W. Cornforth and G. Popják, *Nature*, **164**, 1053 (1949).

¹⁶F. A. Loewus, F. H. Westheimer and B. Vennesland, *J. Am. Chem. Soc.*, **75**, 5018 (1953).

ACETALDEHYDE-H₂

J. E. Zanetti and D. V. Sickman, *J. Am. Chem. Soc.*, **58**, 2034 (1936).

A. Procedure

About 1 g. of dry mercuric sulfate is placed in an all-glass absorption vessel, equipped with a shaking mechanism (Note 1), and connected to a 1-l. gas reservoir containing acetylene-H₂ over mercury. A tube containing approximately the needed amount of phosphorus pentoxide is sealed to a side-tube on the absorption vessel, the apparatus is evacuated to dry it, and 25 to 30 g. of phosphorus pentoxide is volatilized into the vessel in a current of dry oxygen. A tube containing 25 g. of water-H₂ is

then sealed to the system, and the water is distilled into the reaction vessel under a pressure of less than 1 mm. (Note 2). The final absorbing solution is 40-50% phosphoric acid- H_2^2 in water- H_2^2 with 1 g. of mercuric sulfate catalyst. Acetylene- H_2^2 is introduced into the solution from the reservoir; the reaction rate is greatly increased by vigorous agitation (Note 3).

When acetylene absorption ceases, the reaction mixture is distilled *in vacuo* into a flask cooled with liquid air. Acetylene- H_2^2 , acetaldehyde- H_4^2 , water- H_2^2 and paraldehyde- H_{12}^2 are collected. The distillate is fractionated under high-vacuum using Dry Ice-acetone baths, at various temperatures, and liquid nitrogen to effect the fractionation. About 50 g. of acetaldehyde- H_4^2 and 6 g. of paraldehyde- H_{12}^2 are obtained (Note 4). After numerous fractionations, by bulb-to-bulb distillation under vacuum, the acetaldehyde- H_4^2 (Note 5) is obtained as a water-white liquid, b.p. 20.5° (756 mm.), m.p. -121.7° , $d_4^{20} 0.883$, vapor pressure 327 mm. at 0° . Chemically, the acetaldehyde- H_4^2 behaves as ordinary acetaldehyde (Note 6).

Paraldehyde- H_{12}^2 , a by-product in the preparation which floats on top of the water- H_2^2 fraction, is collected, dried with Drierite and fractionally distilled. The paraldehyde, b.p. $124-125^\circ$ (753 mm.), is obtained as a water-white oily liquid, which solidifies below 13° and crystallizes in long prismatic needles, m.p. 13.7° .

B. Notes

1. Zanetti and Sickman show a diagram of the apparatus, which includes a generator for preparing acetylene- H_2^2 from calcium carbide; see Figure XVI, 3.

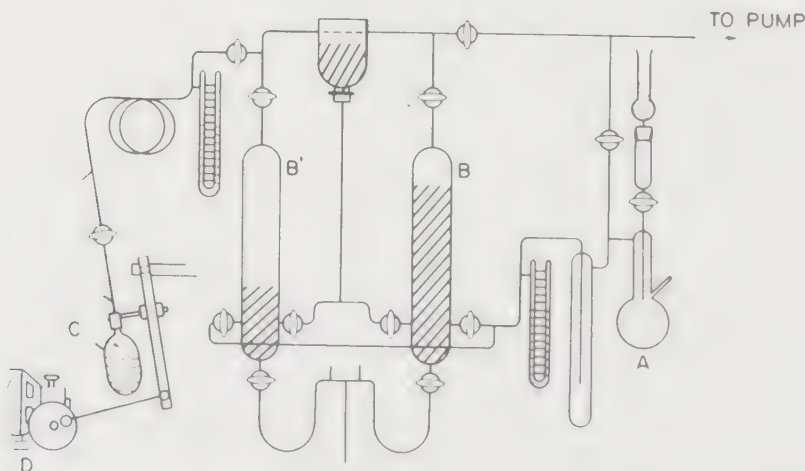


Fig. XVI, 3 Apparatus for preparation of acetaldehyde- H_4^2 (J. E. Zanetti and D. V. Sickman). A, acetylene generator; B and B', mercury reservoirs; C, reaction vessel; D, shaking device with motor and eccentric.

2. Every precaution was taken to prevent contamination with moisture from the air and also from the oxygen torch used in making seals. Stop-cocks were suitably arranged so that any moisture formed could be pumped out without coming in contact with reaction materials.

3. The initial rapid absorption of acetylene- H_2^2 , 1 l. in 20 minutes, gradually reduces to 1 l. per hour.

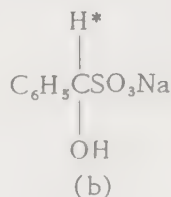
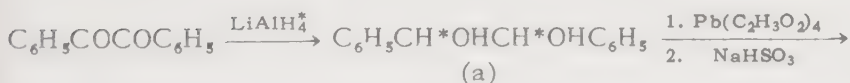
4. The residue in the reaction vessel is treated with anhydrous sodium carbonate and potassium permanganate to recover the deuterium as water- H_2^2 .

5. The final product contained 99.2 atom per cent deuterium as compared to 99.6 atom per cent in the water- H_2^2 used in the synthesis.

6. The acetaldehyde- H_4^2 ammonia compound forms in small white cubes, m.p. $92-94^\circ$ (dec.).

SODIUM α -HYDROXY- α -TOLUENESULFONATE- α - H^2
(Benzaldehyde- H^2 Sodium Bisulfite Addition Compound)

METHOD I



K. B. Wiberg, J. Am. Chem. Soc., 76, 5371 (1954).

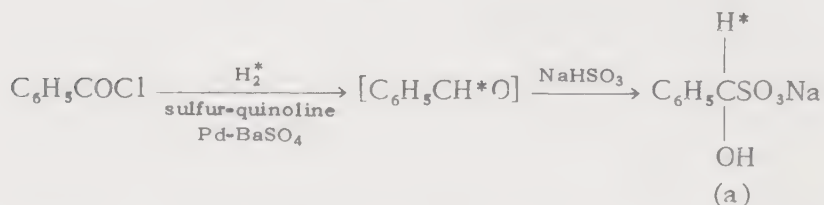
A. Procedure

(a) *Hydrobenzoin- α, α' - H_2^2* , (1,2-Diphenyl-1,2-ethanediol-1,2- H_2^2). To a mixture of 1.0 g. (0.025 mole) of lithium aluminum hydride- H_4^2 in 50 ml. of anhydrous ether (Note 1) is added, with stirring, a solution of 10 g. (0.05 mole) of benzil in about 100 ml. of ether. After one hour, the mixture is treated with a 30% solution of potassium sodium tartrate (Rochelle salt). The ether layer is separated, and the residue is extracted with warm ether. The combined ether solution is dried over sodium sulfate. After the ether is removed, the yield of hydrobenzoin- α, α' - H_2^2 is 10 g.

(b) *Sodium α -Hydroxy- α -toluenesulfonate- α - H^2* . To a solution of 6.0 g. (0.028 mole) of hydrobenzoin- α, α' - H_2^2 in 150 ml. of benzene is added 13 g. (0.29 mole) of lead tetraacetate, with shaking. After 1 hour, water is added, the mixture is filtered, and the benzene layer is separated. Most of the benzene is removed by distillation, and the residual benzaldehyde- H^2 is converted to the bisulfite addition compound with 40% sodium bi-

sulfite solution. After the produce is collected and washed with ether, the yield is 9 g. (75%) (Note 2).

METHOD II



A. F. Thompson, Jr. and N. H. Cromwell, J. Am. Chem. Soc., 61, 1374 (1939).

A. Procedure

(a) *Sodium α -Hydroxy- α -toluenesulfonate- α -H²*. A mixture of 6 g. of benzoyl chloride, 25 ml. of xylene, 10 mg. of Rosenmund's¹ sulfur-quinoline, and 2 g. of 5% palladium-on-barium sulfate catalyst is heated to 145°. Hydrogen-H₂² is circulated through the reaction mixture for 9 hours (Note 3). The total hydrogen-H₂² consumed is 150 ml. After filtering to remove the catalyst, the mixture is shaken with sodium bisulfite solution for 15 minutes, and the bisulfite addition compound is collected by centrifugation, washed with ether and dried. The yield is 1.87 g., which is 66% based on hydrogen-H₂² consumed. Free aldehydes apparently do not exchange aldehyde deuterium with solvent water (Note 4), but the benzaldehyde-H² bisulfite addition compound exchanges 76.5% of its deuterium in 412 hours.

(b) *Sodium α -Hydroxy-4-biphenylmethanesulfonate- α -H², (p-Phenylbenzaldehyde-H² Sodium Bisulfite Addition Compound)*. A mixture of 6 g. of p-phenylbenzoyl chloride, 3 g. of 5% palladium-on-barium sulfate and 10 mg. of sulfur-quinoline is placed in 30 ml. of xylene, and hydrogen-H₂² is passed through the mixture for 11 hours at 140°. The total hydrogen-H₂² consumption is 165 ml. The yield of bisulfite addition compound, prepared in the same manner as the benzaldehyde-H² addition compound, above, is 2 g. (47%).

(c) *p-Phenylbenzaldehyde-1-H²*. p-Phenylbenzaldehyde-1-H² sodium bisulfite addition compound, 2 g., is treated with 3% sodium carbonate, and the free aldehyde is collected and recrystallized from ligroin. The yield of p-phenylbenzaldehyde-1-H², m.p. 55-56°, is 1 g.

B. Notes

1. The ether was distilled from sodium hydride just before use.
2. Benzaldehyde-H² was obtained from the addition compound with 10% sodium carbonate solution immediately before use.

3. Thompson and Cromwell developed an apparatus, which is illustrated in the original literature, for carrying out the reduction with a minimum of hydrogen- H_2^2 which is recycled by means of a glass pump described by Livingston.² Unfortunately it is not possible to prepare a pure H^2 -aldehyde in this manner because of hydrogen-deuterium exchange under the experimental conditions employed.

4. Thompson and Cromwell found no exchange between *p*-phenylbenzaldehyde- H^2 and water during 412 hours. This is to be expected in view of the work of various investigators.³⁻⁵ It is surprising that no exchange of aldehyde hydrogen with the solvent occurs in the Cannizzaro reaction in water- H_2^2 .⁶ Apparently hydrogen is transferred directly from an oxidized molecule to one being reduced without participation of the solvent.

C. Other Preparations

Hydrobenzoin- α, α' - H_2^2 , 3, 4, 3', 4'-bis(methylenedioxy)hydrobenzoin- α, α' - H_2^2 , 4,4'-dichlorohydrobenzoin- α, α' - H_2^2 , benzaldehyde- H^2 , piperonal- H^2 and 4-chlorobenzaldehyde- H^2 have been prepared⁷ from the properly substituted benzil according to the procedure described under Method I. In the case of piperil and 4,4'-dichlorobenzil, low solubility in ether precluded the addition of their ether solutions to lithium aluminum hydride- H_2^2 solutions. Therefore, the substituted benzil, in each case, was placed in the thimble of a Soxhlet extractor and extracted into the hydride solution with ether.

¹K. W. Rosenmund and F. Zetsche, *Ber.*, 54, 425 (1921)

²R. Livingston, *J. Phys. Chem.*, 33, 955 (1929).

³H. F. Bonhoeffer and K. Wirtz, *Z. physik. Chem.*, B32, 108 (1936).

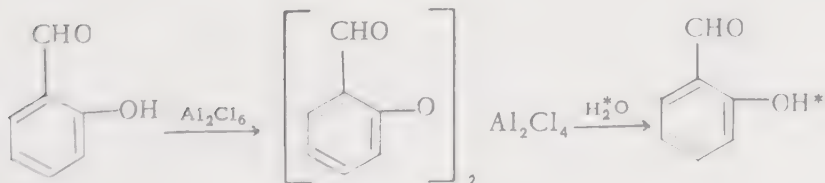
⁴M. Harada and T. Titani, *Bull. Chem. Soc. Japan*, 11, 465 (1936).

⁵K. F. Bonhoeffer and H. Fredenhagen, *Z. physik. Chem.*, A181, 379 (1938).

⁶K. F. Bonhoeffer and H. Fredenhagen, *Naturwissenschaften*, 25, 459 (1937).

⁷K. B. Wiberg and R. Stewart, *J. Am. Chem. Soc.*, 77, 1786 (1955).

o -HYDROXY- H^2 -BENZALDEHYDE (H^2 -Salicylaldehyde)

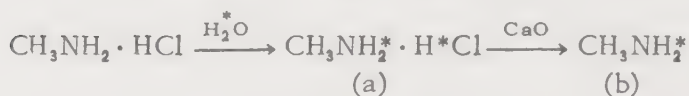


G. B. Bonino and R. Manzoni-Ansidei, *Ricerca sci.*, 9, II, 470 (1938); through *Chem. Abstracts*, 33, 9138 (1939).

Procedure

By treating *o*-hydroxybenzaldehyde with aluminum chloride, the complex, $(\text{OC}_6\text{H}_4\text{CHO})_2\text{Al}_2\text{Cl}_4$, is obtained as a yellow powder. The powder is suspended in absolute ether and treated with water- H_2^2 . When the complex is completely destroyed, the product is extracted with anhydrous ether and fractionated under reduced pressure after removal of ether.

METHYLAMINE- N-H_2^2



H. J. Emeleus and H. V. A. Briscoe, *J. Chem. Soc.* 1937, 127, E. R. Roberts, H. J. Emeleus and H. V. A. Briscoe, *ibid.*, 1939, 41.

A. Procedure (Note 1)

(a) *Methylamine- N-H_2^2 Hydrochloride- H^2* . A tube containing a quantity of water- H_2^2 (99.5%), sealed in a vacuum, and a reaction vessel containing 0.6 g. of methylamine hydrochloride are attached to the vacuum system. When the apparatus is evacuated (Note 2), successive quantities of water- H_2^2 : 0.65, 0.73, 0.70, 0.68 and 0.55 ml., are distilled into a graduated tube for measurement and then, in turn, into the reaction vessel containing the amine hydrochloride. The water- H_2^2 is, in each case, warmed to $30\text{--}35^\circ$ for 30 minutes and then distilled into a storage flask (Note 3). The residue from the last distillation is methylamine- N-H_2^2 hydrochloride- H^2 .

(b) *Methylamine- N-H_2^2* . After admission of dry air into the reaction vessel, an excess of freshly ignited calcium oxide is introduced, and the mixture of lime and amine hydrochloride is heated at $150\text{--}200^\circ$ in a vacuum. The evolved amine is collected over calcium oxide in a U-tube cooled with liquid air. To remove traces of ammonia, the amine is distilled, *in vacuo*, from a bath at -90° (Note 4) into two U-tube receivers in series maintained respectively at -110 to -115° and -190° . The methylamine- N-H_2^2 condenses almost entirely in the former tube at -110 to -115° ; ammonia passes into the colder receiver. The fractionation process is repeated a second time (Note 5).

(c) *Ethylamine- N-H_2^2 Hydrochloride- H^2* . Ethylamine- N-H_2^2 hydrochloride- H^2 is prepared by exchange with water- H_2^2 according to the above procedure for methylamine- N-H_2^2 (Note 6). Ethylamine hydrochloride, 0.760 g., is treated with 6 successive portions of water- H_2^2 in the ratio of 3 moles of water per mole of salt.

(d) *Ethylamine- N-H_2^2* . The free amine, liberated from the salt and partially dried by the action of calcium oxide, is distilled under vacuum

from a bath at -78° and collected at -110° (Note 7). The boiling point of the purified product is 17.5° ; m.p. -78.5° .

(e) *Dimethylamine-N-H² Hydrochloride-H²*. The isotopic compound is prepared from 1.4 g. of inactive amine salt by the above exchange process, using 2 moles of water-H₂² to 1 mole of the amine hydrochloride in each step.

(f) *Dimethylamine-N-H²*. The free dimethylamine-N-H² is liberated from the above hydrochloride and partially dried with calcium oxide prior to further drying with metallic sodium. The product is distilled under vacuum from a bath at -78° into a receiver cooled to -115° (Note 8). The boiling point of the final product is 7.75° ; m.p. -91.1° .

B. Notes

1. All the reactions and the transfer of reagents and the product were carried out in a vacuum system. This is a general procedure, and the preparation of methylamine-N-H₂² is given as an example.

2. As shown in a diagram of the apparatus, Emeleus and Briscoe used an irregular glass rod, attached to a stopcock, to break the capillary seal on the water tube.

3. With water-H₂² of 99.5% isotope content, calculations show that 99.2% of the replaceable hydrogen should be exchanged by this treatment.

4. Traces of water, carbon dioxide and less volatile impurities are retained.

5. Emeleus and Briscoe fractionated the methylamine-N-H₂² still further for use in comparative vapor pressure and absorption spectra studies. The vapor pressures of methylamine-N-H₂² are consistently less than those of methylamine, at various temperatures, by an amount which varies from 5.4 mm. at -60° to 31.8 mm. at -10° . The boiling points of methylamine-N-H₂² and methylamine, as read from a $\log p$ vs. $1/T$ graph, are -5.1° and -6.1° , respectively; extrapolation of vapor pressure curves gave, respectively, $5.2 \pm 0.1^{\circ}$ and $6.2 \pm 0.1^{\circ}$. The melting points of the deuterio and ordinary amine are, respectively, -89.2° and -93.1° .

6. Because of the deliquescent nature of ethylamine hydrochloride, the weight of the salt taken was obtained after evacuation of the sample to constant weight.

7. Because of incomplete drying, it was necessary to distill the amine 4 times to get a constant vapor pressure curve.

8. Three distillations under these conditions gave a product with a constant vapor pressure curve.

C. Other Preparations

In studying the influence of nitro compounds on the exchange of hydrogen-H₂² with acetic acid in the presence of a platinum catalyst, Line¹

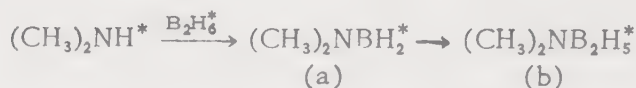
prepared ethylamine- $N\text{-H}_2^2$ from nitroethane.

Dimethylamine- $N\text{-H}^2$ has been prepared² by the reaction of bis(dimethylamino)borine with water- H_2^2 at room temperature. The vapor tension of this product was 546.6 mm. (cor.) at 0° .

¹L. E. Line, Jr., B. Wyatt and H. A. Smith, J. Am. Chem. Soc., 74, 1808 (1952).

²A. B. Burg, *ibid.*, 74, 1340 (1952).

DIMETHYLAMINODIBORANE- H_5^2



A. B. Burg, J. Am. Chem. Soc., 74, 1340 (1952).

A. Procedure

(a) *Dimethylaminoborine- H_2^2* . A mixture of 112.2 cc. (S.T.P.) of diborane- H_6^2 and 203.7 cc. (S.T.P.) of dimethylamine- H^2 is heated in a 300-cc. bulb at 137° for 6 hours. After separation of the more volatile by-product, dimethylaminodiborane- H_5^2 , the dimethylaminoborine- H_2^2 , which is obtained in 90% yield, melts at $74.3\text{--}74.5^\circ$ (Note 1).

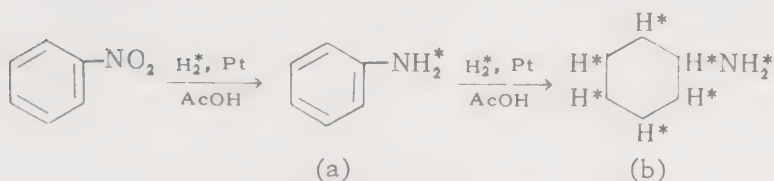
(b) *Dimethylaminodiborane- H_5^2* . Dimethylaminoborine- H_2^2 is treated with more diborane- H_6^2 at 106° for 12 hours. Dimethylaminodiborane- H_5^2 forms quantitatively and has a vapor tension of 105.2 mm. at 0° ; m.p. -57.0° (Note 2).

B. Notes

1. Burg and Randolph¹ describe the purification of dimethylaminoborine by means of a fractionating column operating at 98° and 400 mm. pressure (dry nitrogen) and delivering into a vacuum system. The elevated temperature and the pressure are necessary to prevent solidification of the product during the process. The product may be purified still further by recrystallization from petroleum ether. The product, m.p. $74.5\text{--}75^\circ$, has a vapor tension of 9.1 mm. at 23° .

2. The melting point reported¹ for ordinary dimethylaminodiborane was -54.6° ; the vapor tension was 101.4 mm. at 0° .

¹A. B. Burg and C. L. Randolph, Jr., J. Am. Chem. Soc., 71, 3451 (1949).

CYCLOHEXYLAMINE-*N,N,1,2,3,4,5,6-H*₈²

L. E. Line, Jr., B. Wyatt and H. A. Smith, J. Am. Chem. Soc., 74, 1808 (1952).

A. Procedure (Note 1)

(a) *Aniline-N-H*₂². The hydrogenation of nitrobenzene with hydrogen-*H*₂² is carried out in a modified Parr low-pressure apparatus (Note 2). In a typical experiment, 1-3 ml. of nitrobenzene in 50 ml. of acetic acid is hydrogenated at 30° in the presence of 0.0052 g. of platinum oxide catalyst,¹ with an initial hydrogen pressure of 25 p.s.i.a. Reduction of the nitro group is rapid, the theoretical hydrogen uptake being realized in a few minutes (Note 3).

(b) *Cyclohexylamine-N,N,1,2,3,4,5,6-H*₈². A solution of 3 ml. of aniline-*N-H*₂² in 50 ml. of acetic acid, containing 0.08 g. of platinum oxide catalyst, is hydrogenated at 30°, with an initial hydrogen pressure of 27 p.s.i.a. (Notes 4 and 5).

B. Notes

1. The exchange between hydrogen-*H*₂² and acetic acid in the presence of Adams platinum catalyst¹ is completely suppressed by small amounts of nitrobenzene. However, the nitrobenzene is hydrogenated, first yielding aniline and then cyclohexylamine. Experimental data are taken, in part, from the work of Smith and Bedoit.²

2. The 4-1. hydrogen tank may be replaced with a small tank (about 1 l.) and a more sensitive gauge which is used to follow hydrogen-*H*₂² uptake.

3. The aromatic nitro group is extremely reactive so that a very small amount of the catalyst is necessary. In a similar experiment with 0.085 g. of platinum catalyst, Smith and Bedoit² found the nitro group to be completely reduced in about 6 minutes.

4. As shown by the data of Smith and Bedoit,² the rate of hydrogenation of the benzene ring is about 1/22 of the rate for the nitro group. Under the conditions given aniline is 65% reduced to cyclohexylamine in 36 minutes.

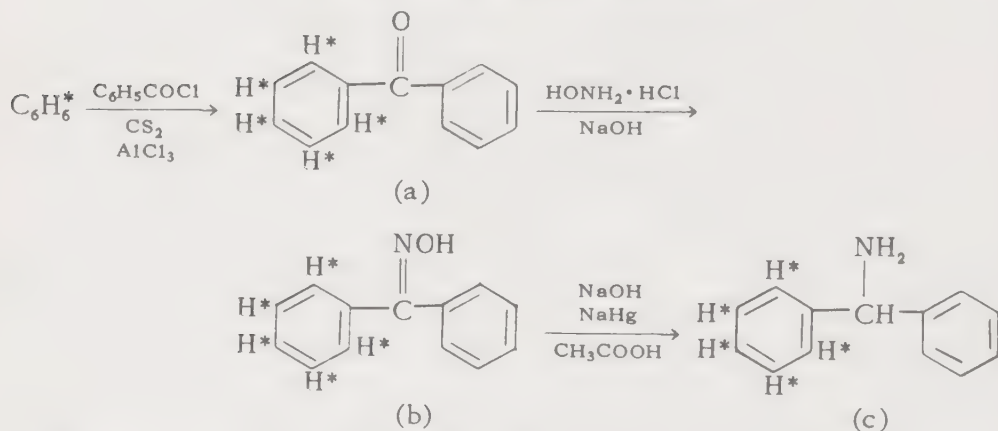
5. Once the nitro group is reduced in the reduction of aniline, the hydrogen-*H*₂² will be diluted with hydrogen by exchange with acetic acid.

If no dilution of the hydrogen- H_2^2 is wanted, acetic acid- H^2 should be used for the solvent.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

²H. A. Smith and W. C. Bedoit, *J. Phys. Colloid Chem.*, 55, 1085 (1951).

α -PHENYL- H_5^2 -BENZYLAMINE



G. R. Clemo and A. McQuillen, *J. Chem. Soc.*, 1936, 808.

A. Procedure

(a) *Benzophenone-2,3,4,5,6- H_5^2* . Benzene- H_6^2 , 0.6 g., is placed in a small flask (3 ml.), fitted with a short condenser tube, which is cooled at the bottom by a water-condenser and at the top by a jacket containing solid carbon dioxide. To the benzene is added 1 ml. of carbon disulfide and 0.75 g. of powdered aluminum chloride. The mixture is cooled in Dry Ice, 0.8 g. of benzoyl chloride is added, the condenser is attached, and the mixture is refluxed for 12 hours. A trap containing potassium hydroxide is attached directly to the condenser to collect the evolved hydrogen- H^2 chloride. The aluminum chloride complex is decomposed by boiling with a solution of 1 ml. of hydrochloric acid in 5 ml. of water. The product is extracted into ether, washed with dilute sodium hydroxide solution and water, and dried over sodium sulfate. After removal of ether, 0.95 g. of benzophenone-2,3,4,5,6- H_5^2 distills at 160° (15 mm.) (Note 1).

(b) *Benzophenone-2,3,4,5,6- H_5^2 Oxime*. Benzophenone-2,3,4,5,6- H_5^2 , 0.95 g., in 9 ml. of alcohol is heated under reflux for 2 hours on a water-bath after the addition of 1.1 g. of hydroxylamine hydrochloride in 2.7 ml. of water and 1.8 g. of potassium hydroxide in 2.7 ml. of water. The solution is poured into 12 ml. of water and acidified with dilute sulfuric acid. The product, 0.95 g., is collected and recrystallized from alcohol; m.p. 140° (Note 2).

(c) α -Phenyl- H_5^2 -benzylamine. Benzophenone-2,3,4,5,6- H_5^2 oxime, 0.95 g., is reduced with 150 g. of 4% sodium amalgam in dilute alkaline solution during 6 hours of heating on a water-bath. Dilute acetic acid is added occasionally to reduce the concentration of alkali. The base is extracted with 5 portions of ether and dried over sodium sulfate. After removal of ether, the free base (0.89 g.) is obtained as a yellow oil (Note 3).

(d) α -Phenyl- H_5^2 -benzylamine D-Hydrogen Tartrate. An aqueous solution of 0.7 g. of D-tartaric acid is added to 0.89 g. of α -phenyl- H_5^2 -benzylamine and evaporated to dryness. The acid tartrate salt, 1.2 g., is then recrystallized from a mixed solvent of alcohol and petroleum ether. Fine needles, m.p. 181° , are formed (Note 4).

(e) α -Phenyl- H_5^2 -benzylamine Oxalate. To 0.16 g. of phenyl- H_5^2 -benzylamine is added an aqueous solution of 0.06 g. of oxalic acid dihydrate. The oxalate salt, 0.16 g., melts at 204° after recrystallization from water (Note 5).

B. Notes

1. Adams and Tarbell¹ prepared benzophenone-2,3,4,5,6- H_5^2 according to the procedure of Clemo and McQuillen but found it necessary to heat the mixture only 1 hour rather than 12 hours. They report the melting point to be $47-48^\circ$.

2. The melting point was not depressed by admixture with authentic benzophenone oxime. Adams and Tarbell¹ report a melting point of $142-143^\circ$.

3. The product rapidly absorbs carbon dioxide.

4. Clemo and McQuillen reported a rotation of $[\alpha]_D + 13.2^\circ$ for the D-tartrate and -13.1° for the L-tartrate after 10 recrystallizations of each fraction. These rotations, as compared to the normal rotation of the D- and L-tartrates of nonisotopic 1,1-diphenylmethylamine, $\pm 12.3^\circ$, together with rotations of $[\alpha]_D^{16} -5.7^\circ$ and $+5.0^\circ$, respectively, for the two forms of the isotopic amine, were taken as evidence of an asymmetric carbon atom in α -phenyl- H_5^2 -benzylamine. Adams and Tarbell¹ were unable to obtain rotations different from those of the nonisotopic compounds and suggested that the impure benzene- H_6^2 , m.p. -1° , used by Clemo and McQuillen might be at fault. Clemo and Swan² repeated the work with pure benzene- H_6^2 and were unable to resolve the α -phenyl- H_5^2 -benzylamine into optically active forms.

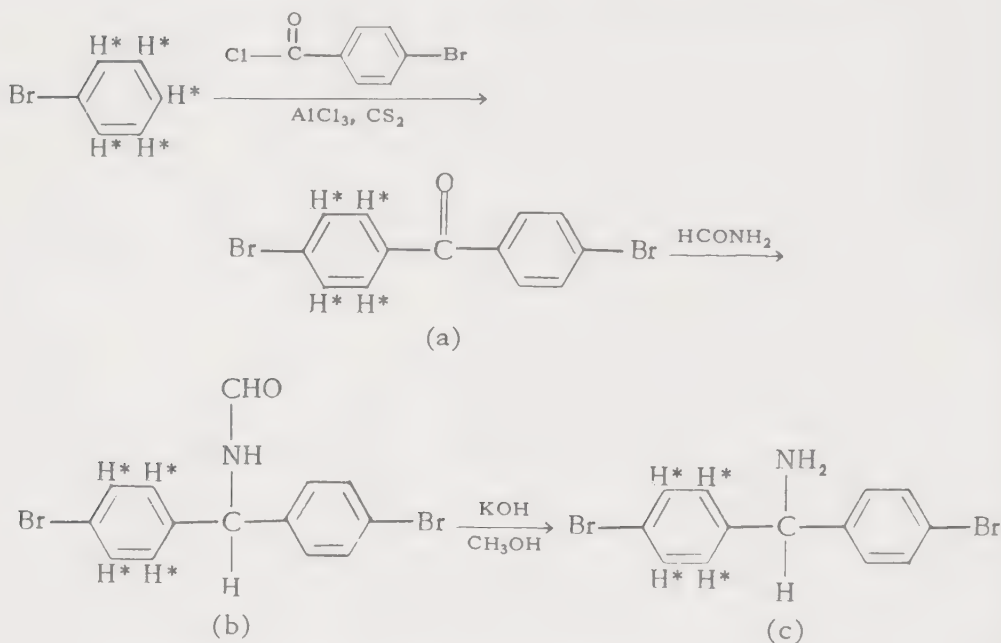
5. Adams and Tarbell¹ did not prepare the oxalate salt of α -phenyl- H_5^2 -benzylamine but did prepare the hydrochloride, m.p. 290° (dec.), by passing dry hydrogen chloride into a solution of the amine in ether. They also prepared α -phenyl- H_5^2 -benzylamine D-bromocamphorsulfonate from a mixture of 2.48 g. of the amine hydrochloride in 25 ml. of water and 3.62

g. of the ammonium salt of D-bromocamphorsulfonic acid dissolved in 20 ml. of water. After crystallization of the precipitate from 100 ml. of water, 4.71 g. of the salt is obtained, m.p. 237-239° (dec.)

¹R. Adams and D. S. Tarbell, J. Am. Chem. Soc., 60, 1260 (1938).

²G. R. Clemo and G. A. Swan, J. Chem. Soc., 1939, 1960.

4-BROMO- α -(4-BROMOPHENYL- H_4^2)BENZYLAMINE



G. R. Clemo and G. A. Swan, J. Chem. Soc., 1942, 370.

A. Procedure

(a) *4,4'-Dibromobenzophenone-2,3,5,6- H_4^2* . In a small flask, fitted with an all-glass condenser which is attached to a trap containing potassium hydroxide, are mixed 0.4 g. of bromobenzene- H_5^2 , 0.55 g. of 4-bromobenzoyl chloride, 0.38 g. of freshly sublimed aluminum chloride and 0.3 ml. of dry carbon disulfide. The mixture is heated 22 hours on the water-bath, cooled, treated with ice and dilute hydrochloric acid, and warmed on the water-bath for 10 minutes to effect hydrolysis of excess acid chloride. The ketone is extracted into chloroform, washed with water and aqueous sodium hydroxide, and dried over sodium sulfate. After removal of the solvent, the product is recrystallized from ethanol. The yield of colorless leaflets is 0.55 g., m.p. 172-173°.

(b) *2-(4-Bromophenyl)-2-(4-bromophenyl- H_4^2)acetamide*. The 0.55 g. of 4,4'-dibromobenzophenone-2,3,5,6- H_4^2 is heated with 1.7 ml. of formamide at 175° for 6 hours. After the solution is poured into water, the product is collected, washed with water, dried and recrystallized from methanol. The

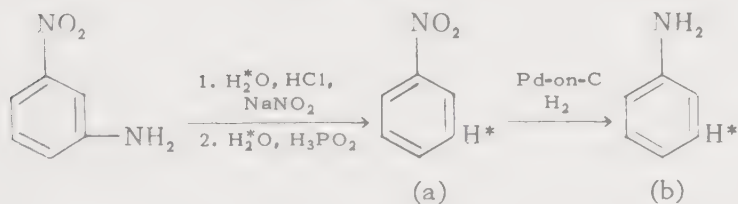
yield of colorless prisms, m.p. $158-159^{\circ}$, is 0.50 g.

(c) 4-Bromo- α -(4-bromophenyl- H_4^2)benzylamine. The amide, 0.50 g., is heated under reflux with a solution of 0.7 g. of potassium hydroxide in 4 ml. of methanol for 3.5 hours on the water-bath. After distillation of most of the methanol, the residue is poured into water, and the base is extracted into ether and dried over potassium carbonate. After removal of ether, the product is recrystallized from light petroleum (b.p. $60-80^{\circ}$) to obtain 0.37 g. of colorless prisms, m.p. $75-76^{\circ}$ (Note 1).

B. Notes

1. In an unsuccessful attempt to resolve the optical isomers, the D-hydrogen tartrate salt, m.p. $210-212^{\circ}$, $[\alpha]_D^{18} + 9.3^{\circ}$ ($l. = 2$, c , 2.5 in methanol), was prepared. The D-bromocamphorsulfonate, m.p. $260-262^{\circ}$, $[\alpha]_D^{18} + 45.9^{\circ}$ ($l. = 2$, c , 2 in methanol), was also prepared.

ANILINE-3- H^2



E. R. Alexander and R. E. Burge, Jr., J. Am. Chem. Soc., 72, 3100 (1950).

A. Procedure

(a) 1-Nitrobenzene-3- H^2 . To a hot solution of 50 g. (2.5 moles) of water- H_2^2 , 25 ml. of concentrated hydrochloric acid and 27.6 g. (0.2 mole) of 3-nitroaniline is added 55 ml. of concentrated hydrochloric acid. After the mixture is cooled to -5 to 0° , a solution of 14.4 g. (0.2 mole) of 97% sodium nitrite in 35 ml. of water is added dropwise over a period of an hour. To the filtered diazonium salt solution, again cooled to -5° , is added a precooled solution of 50.0 g. (2.5 moles) of water- H_2^2 and 66.0 g. (1.0 mole) of anhydrous hypophosphorus acid (Note 1). After the addition, stirring is continued for another hour at -5 to 0° , and the reaction mixture is then placed in a refrigerator for 24 hours.

The reaction mixture is extracted with ether, the combined extracts are evaporated, and, after adding 400 ml. of water, the residue is steam-distilled. The distillate is extracted with two 50-ml. portions of ether, and the extracts are dried over anhydrous magnesium sulfate. After removal of ether, the product is distilled to obtain 10-11.7 g. (41-48%) of 1-nitrobenzene-3- H^2 , b.p. $203-205^{\circ}$ (Note 2).

(b) *Aniline-3-H²*. A solution of 12.3 g. (0.1 mole) of 1-nitrobenzene-3-H² in 100 ml. of alcohol containing 10.7 ml. (0.11 mole) of concentrated hydrochloric acid is hydrogenated at 1-3 atmospheres over 1 g. of 10% palladium-on-carbon catalyst.¹ When the reduction is complete, the catalyst is removed by filtration, and the filtrate is evaporated to dryness *in vacuo*. The yield of aniline-3-H² hydrochloride, m.p. 196-198°, is 98% (Note 3).

B. Notes

1. Prepared by heating 50% aqueous hypophosphorous acid *in vacuo* at 30° until no more water could be removed.

2. It was reported earlier² that the deamination of *m*-nitrobenzene-diazonium chloride by hypophosphorous acid in water-H₂² solution introduced no deuterium into the aromatic nucleus. This was based upon an analysis by infrared spectroscopy. Mass spectrometric analysis of the sample of 1-nitrobenzene-3-H² obtained in this work indicated the presence of 2.55 atom % excess deuterium. Yet, infrared analysis again showed the absorption curve of the sample to be identical with that of ordinary nitrobenzene in the region of 2000-2300 cm⁻¹.

3. Mass spectrometric analysis indicated 1.62 atom per cent excess deuterium. Since the value calculated on the basis of no hydrogen-deuterium exchange during the hydrogenation was 1.60, probably no exchange took place. The infrared absorption curve of this sample in the region 2000-2300 cm⁻¹ was identical with that of aniline hydrochloride.

C. Other Preparations

1-Nitrobenzene-3-H² and aniline-3-H² have been prepared³ essentially according to the procedure described. By preparing compounds of a higher deuterium content, it was shown, in contrast to the earlier work, that these compounds did possess infrared absorption bands in the characteristic C-H² stretching vibration range which are not present in the spectra of the corresponding undeuterated compounds. The positions of the bands were: 2285 ± 3 cm.⁻¹ for 1-nitrobenzene-3-H² and 2270 ± 3 cm.⁻¹ for aniline-3-H², as compared to 2272 ± 3 cm.⁻¹ for benzene-H₁². The intensity of the 1-nitrobenzene-3-H² bond was found to be about half the intensity of the benzene-H₁² bond. Experiments indicated that the intensity of the C-H² stretch in benzene-H₁² is lowered by 30% in nitrobenzene solution and by 20% in triethylamine.

It has been shown⁴ that treatment of 2-bromoaniline with water-H₂² and sodium hydroxide-H² in the presence of Raney nickel-aluminum alloy yields predominantly aniline-2-H². When 3- or 4-bromoaniline is the starting material, mixtures result but the deuterium is located largely at

the 2-position. H^2 -Acetanilide, H^2 -2,4,6-tribromoaniline and H^2 -4'-bromoacetanilide were also prepared.

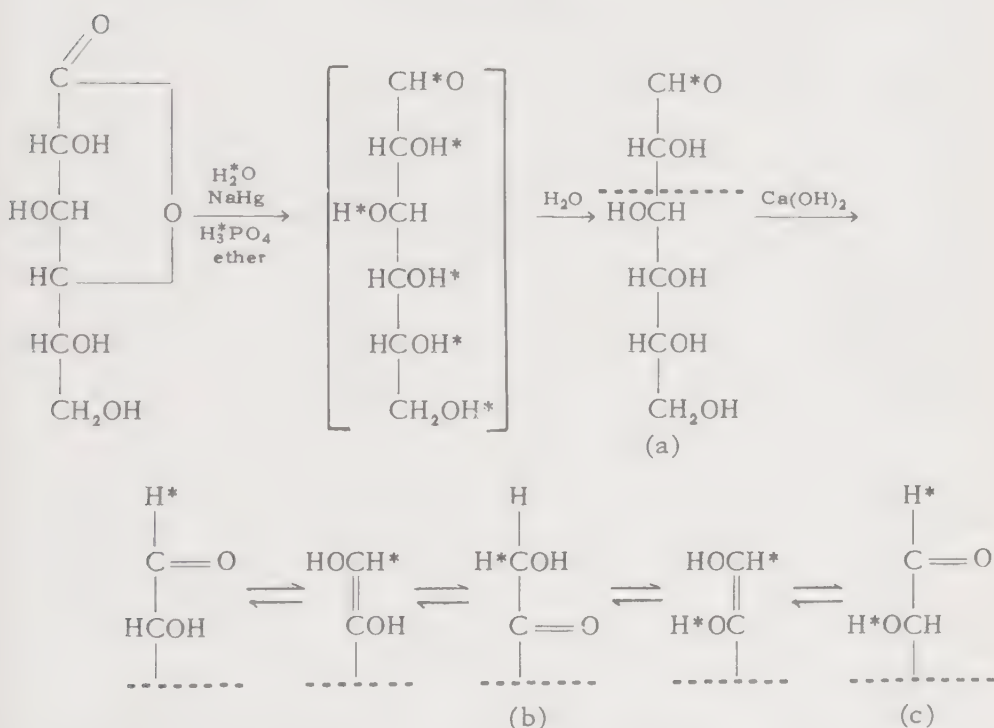
¹*Organic Syntheses*, Vol. 26, Wiley, New York, 1946, p. 78.

²E. R. Alexander and R. E. Burge, Jr., *J. Am. Chem. Soc.*, 70, 876 (1948).

³W. G. Dauben, G. C. Pimentel and C. W. Vaughan, Jr., *ibid.*, 77, 2886 (1955).

⁴W. M. Lauer and L. A. Errede, *ibid.*, 76, 5162 (1954).

GLUCOSE-1- H^2



Y. J. Topper and D. Stetten, Jr., *J. Biol. Chem.*, 189, 191 (1951).

A. Procedure

(a) *Glucose-1- H^2* (Note 1). γ -Gluconolactone, 5 g., (Note 2) is dissolved in 25 g. of water- H_2^2 (99.8%) in a 250-ml. 3-necked flask, fitted with a dropping funnel and a mechanical stirrer. With the temperature of the solution maintained at 5–10°, 90 g. of 2.5% sodium amalgam and a solution of 7 ml. of 85% phosphoric acid- H_2^2 in 15 ml. of dry ether are added (Note 3). Mercury is removed by filtration, and the filtrate is neutralized with solid calcium carbonate. After centrifugation, the solution is made slightly basic (phenolphthalein) by the addition of 0.1 *N* sodium hydroxide (Note 4), then adjusted to pH 6 by the addition of 28% phosphoric acid, and finally neutralized with solid calcium carbonate.

After a second centrifugation, the combined calcium phosphate residues are leached with 100 ml. of hot methanol, and this extract, together with a solution of 8 g. of nonisotopic glucose in 1400 ml. of methanol, is added to the 70 ml. of isotopic glucose solution. After 12 hours a small amount of granular precipitate is removed by filtration, and the filtrate is concentrated to a thick syrup *in vacuo*. Three successive 100-ml. portions of water are added and removed *in vacuo*. The remaining syrup is seeded with D-glucose and permitted to crystallize at 55° in the presence of 50 ml. of ethanol. The crystalline material is collected, washed with cold ethanol and ether, and dried *in vacuo* at 65° (Note 5). The glucose is labeled only in the 1-position (Note 6).

(b) *Fructose-1-H₁²* and (c) *Mannose-1-H²* (Note 7). Saturated lime water is used as the alkaline incubation medium.¹ The solution is approximately 1 M with respect to glucose, and the initial pH is adjusted to 10.6 by the addition of solid calcium hydroxide. Xylene is used as an anti-septic agent. Incubation is terminated when an optical rotation of 3 to 5° is obtained; the solution is adjusted to pH 7 with a small amount of 1 M sulfuric acid. The results from the transformation experiment are given in Table XVI, 6.

TABLE XVI, 6

Treatment of Glucose-1-H² with Saturated Lime Water at 35°.

Compound analyzed	Excess deuterium atom per cent		Deuterium retained (Based on glucose-1-H ²) %
	found	corrected*	
1. Osazone from glucose-1-H ²	1.19	1.37	100
Phenylhydrazone from mannose	0.77	0.77	56
Potassium gluconate	0	0
2. Osazone from glucose-1-H ²	1.33	1.53	100
Phenylhydrazone from mannose	0.82	0.82	54
Osazone from mannose phenyl- hydrazone	0.64	0.80	52
Potassium gluconate	0	0
Osazone from fructose	0.65	0.72	94

* Before final values could be assigned to the deuterium concentration in the products formed from glucose-1-H² during incubation, it was necessary to determine the degree of exchange of carbon-bound deuterium which occurred incidental to the preparation of the isolated derivatives.

(d) *Glucose-1-H² Osazone*. A solution containing 200 mg. of glucose-1-H², 200 mg. of anhydrous sodium acetate, 0.5 ml. of phenylhydrazine, 0.7 ml. of glacial acetic acid and 4 ml. of water is heated on the steam-bath for

30 minutes. The osazone is collected, washed with acetone and recrystallized three times from methanol; m.p. 205° (dec.).

(e) *Mannose-1- H^2 Phenylhydrazone*. Mannose-1- H^2 is isolated from the neutralized incubation solution by precipitation as the phenylhydrazone under conditions precluding osazone formation, i.e., 5° .² The phenylhydrazone is recrystallized three times from 95% ethanol, m.p. $199-200^{\circ}$ (dec.).

(f) *Mannose-1- H^2 Osazone*. A solution containing 200 mg. of mannose-1- H^2 phenylhydrazone, 1.4 g. of phenylhydrazine hydrochloride, 1 g. of sodium acetate trihydrate and 28 ml. of water is refluxed for 30 minutes. After filtration from hot solution the product is washed with acetone and recrystallized three times from methanol, m.p. 205° (dec.).

(g) *Fructose-1- H_1^2 Osazone*. After removal of mannose-1- H^2 , the solution is adjusted to pH 8 with 0.1 *N* sodium hydroxide, and the excess phenylhydrazine is removed by ether extraction. The aqueous solution is then neutralized with 0.1 *N* hydrochloric acid. To an aliquot of this solution, containing 400 mg. of glucose-1- H^2 , is added 200 mg. of uniformly labeled glucose- C_6^{14} . The total glucose content is then oxidized with iodine,³ potassium gluconate- C_6^{14} is collected, and the filtrate is immediately neutralized with 3 *N* hydrochloric acid and evaporated to dryness *in vacuo*. The residue is dissolved in water, a solution of phenylhydrazine hydrochloride and sodium acetate is added, and the resulting solution is heated on a steam-bath for 12 minutes. The product is collected, washed with acetone and recrystallized three times from methanol (Note 8).

B. Notes

1. The method of Sperber⁴ for the reduction of arabinolactone and galactonolactone was adapted to the present isotopic synthesis.

2. This compound is prepared from γ -gluconolactone by the method of Isbell and Frush.⁵

3. The additions are made during one hour in such a manner as to keep the pH between 3 and 4 (Congo red).

4. To hydrolyze unreacted lactone.

5. In two successive preparations the yields, based upon the isotopic content of osazone derived from this material, were 64 and 67%. The reducing power of the glucose, m.p. $142-146^{\circ}$, was 99.1% of that of c.p. anhydrous dextrose, as determined by the method of Cajori.⁶

6. All the deuterium content of the glucose was lost upon oxidation to potassium gluconate.

7. The glucose-1- H^2 was used in studying the Lobry de Bruyn-van Ekenstein⁷ alkali-catalyzed transformation of glucose into fructose and mannose.

8. The complete absence of radioactivity in the osazone indicated that essentially none had been derived from glucose.

C. Other Methods

The isomerization of D-glucose by alkali in water- H_2^2 at 25° has been studied by Sowden and Schaffer.¹ Their results were in accord with the *classical* "enediol" mechanism for the alkaline isomerization of the reducing sugars. D-glucose-1- H^2 , D-fructose-1- H_1^2 and D-mannose-1- H^2 were obtained. A sample of the undiluted deuterated D-fructose was converted to 2,3;4,5-diisopropylidene-D-fructose-1- H_1^2 , m.p. $95-96^\circ$, according to the directions of Pacsu.⁹ Oxidation of the diactonefructose to 2,3; 4,5-diisopropylidene-2-oxogluconic acid with alkaline permanganate, according to the procedure of Ohle,¹⁰ indicated that 92% of the deuterium was located at carbon-1. It was suggested that enolization of D-fructose to a 2,3-enediol introduces the opportunity for formation of some D-fructose labeled with deuterium at carbon-3.

¹M. L. Wolfrom and W. L. Lewis, J. Am. Chem. Soc., 50, 837 (1928).

²E. L. Sherrard and G. W. Blanco, Ind. Eng. Chem., 15, 612 (1923).

³K. P. Link and S. Moore, J. Biol. Chem., 133, 293 (1940).

⁴N. Sperber, H. E. Zaugg and W. M. Sandstrom, J. Am. Chem. Soc., 69, 915 (1947).

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⁶F. A. Cajori, J. Biol. Chem., 54, 617 (1922).

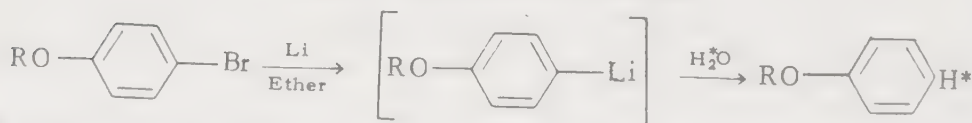
⁷C. A. Lobry de Bruyn and W. A. van Ekenstein, Rec. trav. chim., 14, 203 (1895); 16, 257, 274 (1897).

⁸J. C. Sowden and R. Schaffer, J. Am. Chem. Soc., 74, 505 (1952).

⁹E. Pacsu, E. J. Wilson, Jr., and L. Graf, *ibid.*, 61, 2675 (1939).

¹⁰H. Ohle, I. Koller and G. Behrend, Ber., 58, 2577 (1925); H. Ohle and G. Behrend, *ibid.*, 60, 1159 (1927); H. Ohle and R. Wolter, *ibid.*, 63, 843 (1930).

ALKYL H_1^2 -PHENYL ETHERS



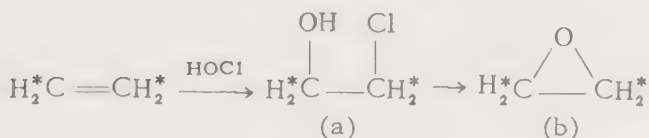
W. M. Lauer and J. T. Day, J. Am. Chem. Soc., 77, 1904 (1955).

Procedure

Several alkyl H_1^2 -phenyl ethers are prepared according to the following general procedure. A solution of 0.1 mole of the 2- or 4-bromophenyl alkyl ether in 75 ml. of anhydrous ether is added to an excess of finely divided lithium (2.0 g.) in 80 ml. of anhydrous ether under nitrogen. The intermediate organolithium compound is formed in a short time at the boiling point of ether. The organolithium compound is decomposed by

the cautious addition of water- H_2^2 . The resulting mixture is filtered to remove lithium bromide and lithium hydroxide. Ethyl ether is removed by distillation, and the residue is then distilled under reduced pressure to obtain the alkyl H_1^2 -phenyl ether. The compounds prepared by this procedure are: anisole-4- H^2 , anisole-2- H^2 , phenetole-4- H^2 , phenyl-4- H^2 propyl ether and phenyl-4- H^2 isopropyl ether.

1,2-EPOXYETHANE- H_4^2
(Ethylene- H_4^2 Oxide)



L. C. Leitch and A. T. Morse, Can. J. Chem., 30, 924 (1952).

A. Procedure

(a) *2-Chloroethanol-1,2- H_4^2* , (*Ethylene- H_4^2 Chlorohydrin*). This compound is prepared by the method of Gomberg.¹ Ethylene- H_4^2 , 0.2 mole, and an equivalent amount of chlorine are passed into water at 0° , with stirring (Note 1). The ethylene- H_4^2 chlorohydrin is not isolated.

(b) *1,2-Epoxyethane- H_4^2* , (*Ethylene- H_4^2 Oxide*). The 5-6% solution of ethylene- H_4^2 chlorohydrin is added slowly to a suspension of 50 g. of calcium hydroxide in 200 ml. of water heated under reflux. The evolved ethylene- H_4^2 oxide is condensed in a trap at -40° which is connected to the top of the condenser. When the solution of chlorohydrin has all been added, the ethylene- H_4^2 oxide is swept out of the reaction flask into the trap with a stream of nitrogen. The product is purified by distillation into a graduated trap cooled to -40° ; the yield is 70%, b.p. 12° (Note 2).

B. Notes

1. Sufficient water is used that the final solution will contain about 5% ethylene chlorohydrin. According to Gomberg,¹ very little ethylene chloride is formed up to this concentration.

2. Analysis by the method of Eastham and Latremouille² indicated 97.5% ethylene- H_4^2 oxide.

C. Other Preparations

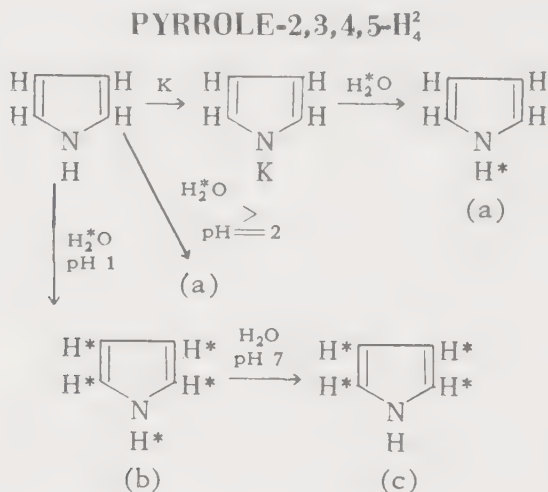
Ethylene- H_4^2 oxide has been prepared³ by the action of KOH on ethylene- H_4^2 chlorohydrin and by passing ethylene- H_4^2 bromide over silver oxide.

Ethylene- H_4^2 sulfide has been prepared³ from ethylene- H_4^2 oxide.

¹M. Gomberg, J. Am. Chem. Soc., 41, 1414 (1919).

²A. Eastham and G. Latremouille, Can. J. Research, 28B, 264 (1950).

³G. L. Cunningham, Jr., A. W. Boyd, R. J. Myers, W. D. Gwinn and W. I. LeVan, J. Chem. Phys., 19, 676 (1951).



F. A. Miller, J. Am. Chem. Soc., 64, 1543 (1942).

A. Procedure

(a) *Pyrrole-1- H^2* . The method employed is that used by Redlich and Stricks¹ and by Bonino and Manzoni-Ansidei² (Note 1). Clean potassium is added slowly, in slight excess, to 10 ml. of freshly distilled pyrrole dissolved in 80 ml. of toluene. The mixture is warmed and later refluxed in a water-free atmosphere until the precipitate becomes white. Then, most of the excess potassium is removed mechanically. The solid is collected on a sintered glass funnel in a gas box, washed with dry ether (Note 2) and dried. The yield of potassiumpyrrole is 80%. The solid is suspended in 40 ml. of dry ether, and water- H_2^2 (99.6%) is added dropwise, with continued shaking, until a second layer of liquid is formed (Note 3). The ether solution of pyrrole-1- H^2 is filtered through a sintered glass funnel and removed from the gas box, and the ether is removed with a stream of dry nitrogen. The product is dried over sodium carbonate and distilled four times, consecutively, in an all-glass apparatus, at low pressure; yield 70% (Note 4).

(b) *Pyrrole- H_5^2* . According to Koizumi and Titani³ pH 1 is optimum for exchanging all five hydrogen atoms of pyrrole (Note 5). Adjusting the water- H_2^2 to pH 1 without diluting the deuterium with hydrogen requires special methods (Note 6). Using water- H_2^2 of this pH, the procedure of

exchange is similar to that for pyrrole-1-H² (see Note 4). The product is dried and then distilled under vacuum.

(c) *Pyrrole-2,3,4,5-H₄²*. This compound is prepared from pyrrole-H₅² by exchange with ordinary water at pH 7. The procedure of exchange and purification is identical with that for pyrrole-1-H².

B. Notes

1. This preparation was repeated because the Raman spectra of the samples^{1,2} differed considerably, and, in addition, pyrrole-1-H² was needed for purposes of comparison.

2. The ether is sufficiently dry to give a blue color with sodium and benzophenone.

3. This required 5.5 ml. of water-H₂², which is about 2.5 times the theoretical amount.

4. Pyrrole-1-H² was also prepared by shaking four 2.5-ml. samples of freshly distilled pyrrole with 2-ml. portions of water-H₂² (99.6%). Each portion of the water-H₂² was used with each of the pyrrole samples in turn. The exchange was continued until the calculated deuterium content was 99% of the *N*-hydrogen. Koizumi and Titani³ suggested that only the *N*-hydrogen in pyrrole exchanges at pH \geq 2. This was verified by comparing the Raman spectra of the two samples of pyrrole-1-H², prepared as described. Although the potassiumpyrrole method is wasteful, the position of the hydrogen-H² atom should be unequivocal.

5. At pH > 1.5, all the hydrogen atoms do not exchange; at pH < 1, acid-induced decomposition of the pyrrole is serious.

6. Hydrogen-H² chloride is made from water-H₂² and thionyl chloride⁴ and is bubbled into water-H₂², to which has been added a trace of dry Methyl violet, until the color of the solution matched that of a comparison solution of hydrochloric acid at pH 1.

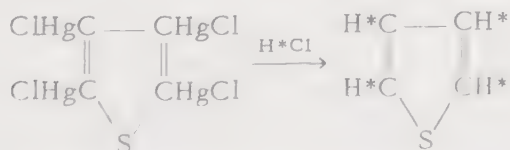
¹O. Redlich and W. Stricks, *Monatsh.*, 68, 47 (1936).

²G. B. Bonino and R. Manzoni-Ansidei, *Ricerca Sci.*, 7, II, 3 (1936).

³M. Koizumi and T. Titani, *Bull. Chem. Soc. Japan*, 13, 85 (1938).

⁴A. Langseth and A. Klit, *Kgl. Danske Videnskab Selskab, Math. fys. Medd.*, 15, No. 13, 7 (1937).

THIOPHENE-H₄²



A. Procedure

Tetrakis(chloromercuri)thiophene, 130 g., is heated with 200 ml. of 18% hydrochloric acid- H^2 in a 2-l. flask (Note 1). The rate of heating is governed by the amount of frothing until the solution is finally heated to boiling. Then, 30 ml. is distilled over into a receiver which contains 4 g. of dry sodium chloride. Moisture is excluded (Note 2), and the mixture is shaken until the sodium chloride dissolves. The two layers are separated, and the thiophene- H_4^2 is dried with freshly heated sodium sulfate and distilled from sodium metal. From four combined preparations are obtained a forerun (b.p. $82.5-82.8^\circ$), 27 g. of main fraction (b.p. $82.8-83.3^\circ$), and a final fraction (b.p. $83.3-84.0^\circ$). The main product melts at -38.83 to -38.54° ; $n_D^{20.7}$ 1.52600; $D_4^{20.0}$ 1.11382 (Note 3).

B. Notes

1. Tetrakis(chloromercuri)thiophene is prepared from tetrakis(acetoxymercuri)thiophene which is obtained from thiophene, according to Paolini and Silberman.¹

2. Cork and rubber stoppers should not be used.

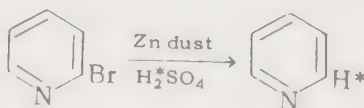
3. According to Schreiner,² pure partially deuterated thiophenes are obtained by treatment of the corresponding chloromercuri-compound with hydrogen- H^2 chloride.

C. Other Preparations

Schreiner² has prepared thiophene- H_4^2 by an exchange reaction with 69% aqueous sulfuric acid- H_2^2 . A homogeneous product, b.p. 82.3° (732.4 mm.), was obtained after 30 hours at 40° or 70 hours at room temperature; $H^2/H \div H^2 = 0.967$, d_4^{20} 1.11220, n_D^{20} 1.5267.

¹O. Paolini and B. Silberman, *Gass. chim. ital.*, 45, II, 385 (1915).

²H. Schreiner, *Monatsh.*, 82, 702 (1951); through *Chem. Abstracts*, 47, 2747 (1953).

PYRIDINE-2- H^2 

B. Bak, L. Hansen and J. Rastrup-Andersen, *J. Chem. Phys.*, 22, 2013 (1954).

A. Procedure

(a) *Pyridine-2- H^2* . To a solution of 0.0085 mole of highly purified 2-bromopyridine in 25 ml. of 2 *N* sulfuric acid- H_2^2 (0.05 equivalents), in a 150-ml. flask fitted with a reflux condenser, is added 1.31 g. (0.05 mole) of zinc dust. With atmospheric moisture excluded, the mixture is kept at

100° for 100 minutes (Note 1). The reaction mixture is cooled and filtered free of excess zinc dust. Then 10–15 ml. of water-H₂² is distilled *in vacuo* from the solution at room temperature into a flask containing a mixture of 5.5 g. of mercuric chloride and 1.5 g. of sodium chloride. The resulting salt solution is added to the pyridine solution with vigorous shaking. The mixture is cooled to 0°, and the white precipitate which forms is collected on a filter and washed twice with ice-cold alcohol and three times with dry ether. Finally, the product is dried *in vacuo* over concentrated sulfuric acid (Note 2). After the complex is dried it is mixed with 2.0 g. of pulverized sodium hydroxide in a 40-ml. tube, which is then evacuated, sealed and heated for 1 hour on a steam-bath. The tube is cooled in liquid air and opened and then is attached to a vacuum line. The liberated pyridine-2-H², contaminated with about 5% water (Note 3), is distilled *in vacuo* from a bath at 10°. Distillation of the product successively from 3 flasks, containing respectively 0.5 g., 0.2 g. and 0.1 g. of pulverized sodium hydroxide, removes the remaining water (Note 4).

Pyridine-4-H² and pyridine-3-H² are obtained, respectively, by the substitution of 4-chloropyridine and 3-bromopyridine for 2-bromopyridine in the above procedure.

B. Notes

1. The amount of hydrogen-H₂² evolved corresponded very closely to the simultaneous occurrence of the reactions $\text{Zn} + \text{H}_2\text{SO}_4 \longrightarrow \text{ZnSO}_4 + \text{H}_2$ and $\text{C}_5\text{H}_4\text{NX} + \text{H}_2 \longrightarrow \text{C}_5\text{H}_4\text{H}^2\text{N} + \text{H}^2\text{X}$. The pyridine is not converted to piperidine.

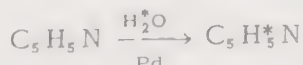
2. The corresponding pyridine complex was first described by Lodenburg,¹ and its composition was proved to be pyridine·HCl·2HgCl₂; m.p. 179°. The isotopic complex obtained by Bak and co-workers had this melting point but not the exact composition shown.

3. Adjudged from the infrared absorption curve.

4. The infrared spectra obtained by Bak, *et al.*, served to show that the above synthesis procedure resulted in the production of pure samples not contaminated with pyridine, isomeric monodeuterated derivatives or polydeuterated derivatives.

¹A. Lodenburg, *Ann.*, 247, 1 (1888).

PYRIDINE-H₅²



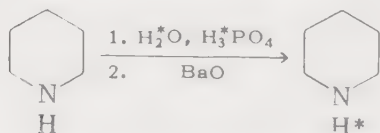
L. Corrsin, B. J. Fax and R. C. Lord, *J. Chem. Phys.*, 21, 1170 (1953)

A. Procedure

The palladium-on-asbestos catalyst is first activated *in situ* with hydrogen- H_2^2 at atmospheric pressure and 240° for 4 hours. The temperature of the exchange vessel is then reduced to 225° . A mixture of pyridine and water vapor, from a distillation flask containing 5 g. of pyridine and 6 g. of water- H_2^2 , is passed over the catalyst. The effluent vapor is condensed and returned to the flask for recycling over the catalyst (Note 1). After equilibrium is reached, the mixture is removed from the apparatus, and most of the water is removed with anhydrous potassium carbonate. A new batch of catalyst is activated, as before, and a new cycle of exchange is carried out. After the cycle is repeated 8 times, the product contains not more than 1 atom per cent hydrogen (Note 2). The final sample amounts to 3.5 ml. (ca. 55%) after it is dried over barium oxide and twice distilled through a micro-fractionating column.

B. Notes

1. The cycling process was usually carried on for 12 hours, although in the later stages the time was extended to 36 hours.
2. Indicated by infrared analysis.

PIPERIDINE-1- H^2 

M. F. Hawthorne, J. Am. Chem. Soc., 76, 6360 (1954).

A. Procedure

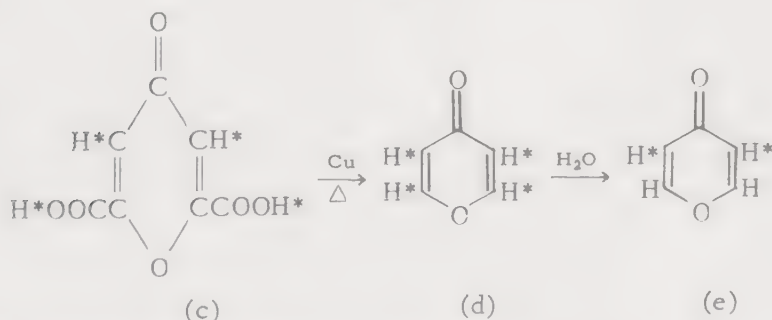
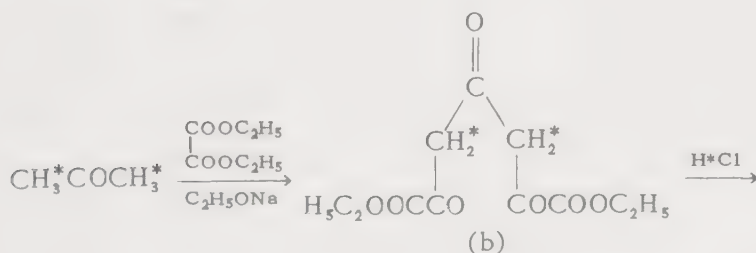
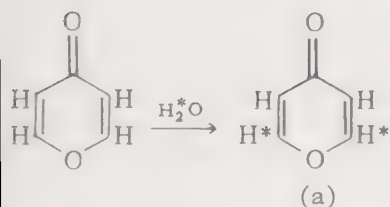
To 17.0 g. of dry piperidine (Note 1) is added 5 ml. of water- H_2^2 and 0.5 ml. of phosphoric acid- H_3^2 (Note 2). The solution is refluxed for 3 hours and then repeatedly distilled from 5-g. batches of barium oxide until the boiling point is that of dry piperidine. The exchange reaction and drying procedure are then repeated once more; the yield of piperidine-1- H^2 , b.p. $104\text{--}105^\circ$, n_D^{25} 1.4502, is 12.2 g. (Note 3).

B. Notes

1. Piperidine was dried by distillation from barium oxide, b.p. $104\text{--}104.5^\circ$, n_D^{25} 1.4509.
2. Phosphoric acid- H_3^2 was prepared by the addition of phosphorus pentoxide to water- H_2^2 .

3. The rates of reaction of 1-chloro-2-nitrobenzene and 1-chloro-4-nitrobenzene with piperidine-1- H^2 in xylene solution at 116° were determined. The corresponding rates were also obtained with ordinary piperidine. The substitution of deuterium for hydrogen did not alter the rates of these reactions, but the *ortho* compound reacts 80 times more rapidly at 116° than the *para* compound. The products were isolated from reactions which had approached completion. The reaction mixtures were rapidly filtered; the products were washed with dry xylene and dried in a desiccator over phosphorus pentoxide. In every instance the colorless, crystalline 1-(2-nitrophenyl)piperidine hydrochloride- H^2 melted at $248-249^\circ$, whereas the corresponding hydrochloride melted at $245-246^\circ$. These melting points were consistently reproducible.

4H-PYRAN-4-ONE-2,6- H_2^2



R. C. Lord and W. D. Phillips, J. Am. Chem. Soc., 74, 2429 (1952).

A. Procedure

(a) 4H-Pyran-4-one-2,6- H_2^2 . 4H-Pyran-4-one (0.1 mole) and 0.5 mole of water- H_2^2 (99.8%) are heated together in a 25-ml. flask fitted with a

reflux condenser and protected by a calcium chloride tube. The mixture is heated with an oil-bath at 95° for 18 hours. The pyranone is separated from the solvent by vacuum distillation. The sample is then heated with another 0.5-mole portion of the water- H_2^2 for an additional 18 hours (Note 1). Exchange of deuterium for hydrogen takes place only at the 2- and 6- positions (Note 2).

(b) *Ethyl 2,4,6-Trioxoheptanoate-3,5- H_4^2* . Chelidonic- H_2^2 acid- H_2^2 is prepared from 0.2 mole of 2-propanone- H_6^2 via ethyl 2,4,6-trioxoheptanoate-3,5- H_4^2 according to the procedure of Riegel and Zwillgmeyer.¹ The crude ethylester is transferred to a 5-cm. Büchner funnel and washed with four 50-ml. portions of distilled water, cooled to 0° , to remove sodium chloride (Note 3). The salt-free ester is dried in a vacuum desiccator over phosphorus pentoxide for several hours; yield, 47 g. (97%).

(c) *4-Oxo-4H-pyran-2,6-dicarboxylic- H_2^2 Acid- H_2^2* , (*Chelidonic- H_2^2 Acid- H_2^2*). To effect the ring closure and hydrolysis of the ester groups, the ethyl 2,4,6-trioxoheptanoate-3,5- H_4^2 is transferred to a 1-l., long-necked flask with calcium chloride tube attached, 60 ml. of concentrated hydrochloric acid- H^2 (Note 4) is added, and the mixture is heated on a steam-bath for 30 hours. After drying for an hour at 100° and 20 mm. and 0.5 hour at 160° and 20 mm., the yield of chelidonic- H_2^2 acid- H_2^2 is 29 g. (79%).

(d) *4H-Pyran-4-one- H_4^2* . *4H-Pyran-4-one- H_4^2* is obtained from chelidonic- H_2^2 acid- H_2^2 by the method described by Willstätter and Pummerer.² The acid is intimately ground with about twice its weight of copper powder and dry distilled at $260-270^{\circ}$. The *4H-pyran-4-one- H_4^2* which distills is collected at room temperature and purified by distillation under vacuum. The yield of product, b.p. 97° (13 mm.), is 5.6 g. (28%).

(e) *4H-Pyran-4-one-3,5- H_2^2* . The 3,5- H_2^2 -compound is obtained by heating *4H-pyran-4-one- H_4^2* (0.037 mole, with 0.074 equivalent of replaceable hydrogen- H^2 at the 2,6-positions) with water (Note 2), at 95° for 25 hours (Note 5). Because of the sensitivity of the pyranone ring to hydrolysis at elevated temperatures, even in neutral solution, about 30% of the pyranone decomposes with each 24 hours of heating under these conditions. Therefore, only 2.2 g. of the mixed product (93% H_2^2 -pyranone, 7% H_4^2 -pyranone) is obtained.

B. Notes

1. The infrared absorption spectrum indicated that the ratio of deuterium to hydrogen in the product was 1:1. Further heating of the partially deuterated pyranone with fresh water- H_2^2 caused no further change in the infrared spectrum.

2. 2,6-Dimethyl-4*H*-pyran-4-one (0.0024 mole) was heated with 99.8% water- H_2^2 (0.25 mole) for 24 hours at 95° . The subsequent infrared

spectrum of the dimethylpyranone indicated that no exchange whatever had occurred, either in the 3- and 5-positions or the methyl groups.

3. The removal of sodium chloride from the ester by washing was of prime importance; otherwise, the yield of chelidonic acid was very small.

4. Concentrated hydrogen- H^2 chloride was prepared by the method of Brown and Groot.³

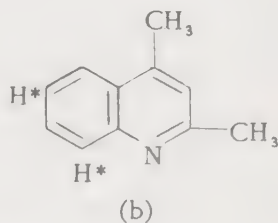
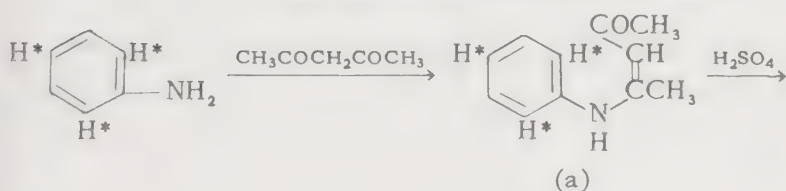
5. The use of one mole of water (1 equivalent of hydrogen) gave a calculated exchange of 93% of the deuterium atoms at the 2- and 6-positions.

¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 126.

²R. Willstätter and R. Pummerer, *Ber.*, 37, 3733 (1904).

³H. C. Brown and C. Groot, *J. Am. Chem. Soc.*, 64, 2223 (1942).

2,4-DIMETHYLQUINOLINE-6,8- H^2



T. G. Bonner and J. M. Wilkins, *J. Chem. Soc.*, 1955, 2358.

A. Procedure

(a) 4-(Anilino-2,4,6- H^2)-3-penten-2-one. The following general method of condensing aniline or substituted anilines with 2,4-pentanedione is that described by Roberts and Turner¹ (Note 1). A mixture of 1 mole of aniline and 1.1 moles of 2,4-pentanedione is gently refluxed for 1-2 hours. The mixture is then cooled and thoroughly shaken with water. Benzene is added, and the two layers are separated. The benzene solution is shaken 3 times with water and is then dried over sodium sulfate. The solvent is removed by distillation and by warming of the product to 100°. 4-(Anilino-2,4,6- H^2)-3-penten-2-one, m.p. 51-53°, is obtained in colorless crystals from petroleum ether.

(b) 2,4-Dimethylquinoline-6,8- H^2 . The cyclodehydration of 4-(anilino-2,4,6- H^2)-3-penten-2-one is according to the following procedure of Bonner, Thorne and Wilkins.² The ketone, 1 g., is rapidly dissolved in

10 ml. of 97.9% sulfuric acid (Note 2), and the solution is kept at room temperature for 2 hours. The solution is then poured into 100 ml. of ice-cold water and left for 2 hours (Note 3). The solution is cooled to 5° and treated with an ice-cold solution of 0.05 g. of sodium nitrite in 5 ml. of water. Any aniline resulting from the hydrolysis is diazotized and is then converted to phenol by warming the solution. The solution is cooled and made alkaline by the addition of 15 g. of sodium hydroxide. The 2,4-dimethylquinoline-6,8- H_2^2 is extracted with ether; the ether solution is washed with water and dried over sodium sulfate. After filtration of the solution and evaporation of the solvent, the crude product is a yellow liquid; yield 92% (Note 4).

B. Notes

1. The formation of substituted quinolines from the condensation products of 2,4-pentanedione with primary arylamines was first investigated by Combes.³ The influence of substituent groups in the aromatic nucleus on the cyclodehydration reaction was examined by Turner and Roberts.¹ Their results together with subsequent work have been summarized by Bradsher.⁴

2. This concentration of ketone was 5 times that used in kinetic studies.

3. Complete hydrolysis of the unreacted ketone was thus assured.

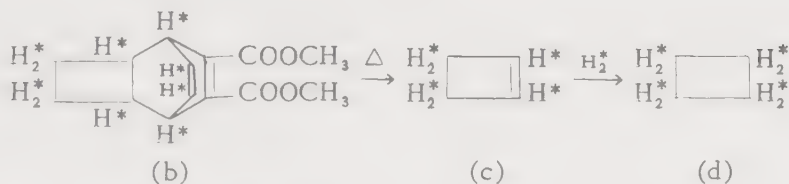
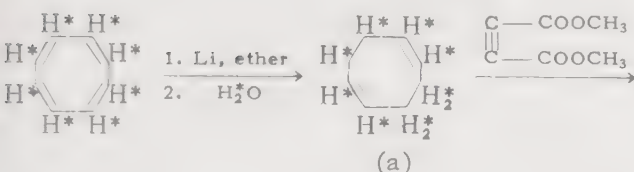
4. In the kinetic study with the isotopic ketone, it was shown by analysis of the product for deuterium content that deuterium exchange with the medium was insignificant. In the cyclodehydration, the isotope effect, measured as the ratio of the reaction rates of 4-(anilino-2,4,6- H_3^2)-3-penten-2-one and 4-anilino-3-penten-2-one, $k_{H_2}:k_H$ was 2:3 in both 95.5% and 89.2% sulfuric acid.

¹E. Roberts and E. B. Turner, *J. Chem. Soc.*, 1927, 1832.

²T. G. Bonner, M. P. Thorne and J. M. Wilkins, *J. Chem. Soc.*, 1955, 2351.

³A. Combes, *Compt. rend.*, 106, 142 (1887); *Bull. soc. chim.*, France, 49, 90 (1888).

⁴C. K. Bradsher, *Chem. Revs.*, 38, 447 (1946).

CYCLOBUTANE- H_8^2 

R. C. Lord, J. Chem. Phys., 21, 378 (1953).

A. Procedure (Note 1)

(a) *1,3,5-Cycloöctatriene- H_{10}^2* . Cycloöctatetraene- H_8^2 is reduced by the addition of lithium in ether solution (Note 2). The resulting organolithium compound is decomposed with water- H_2^2 to obtain 1,3,5-cycloöctatriene- H_{10}^2 (Note 3).

(b) *Methyl Tricyclo[4, 2, 2, 0^{2,5}]deca-7, 9-diene-7, 8-dicarboxylate- H_{10}^2* . A solution of dry 1,3,5-cycloöctatriene- H_{10}^2 (0.0362 mole) in benzene is added to 7.0 g. (0.061 mole) of methyl acetylenedicarboxylate and heated under nitrogen at 60° for 24 hours. The solvent is distilled under reduced pressure, and excess methyl acetylenedicarboxylate is removed by warming the product at 60° and 0.05 mm. during 8 hours. The residual adduct, 7.19 g. (77%), is distilled in a short-path still, b.p. 105° (2 mm.); n_D^{25} 1.5145.

(c) *Cyclobutene- H_6^2* . The above adduct, 4.72 g., is placed in a 5-ml. flask with a capillary inlet attached to a source of dry nitrogen. The flask is attached to a 25-cm. air-cooled condenser leading to a trap cooled with liquid nitrogen. The pressure in the system is reduced to 100 mm., and the flask is heated in a bath at 200° for 20 minutes. The flask is cooled, and nitrogen is admitted. The trap contains 0.97 g. (95%) of cyclobutene- H_6^2 , which is solid at the temperature of liquid nitrogen and liquifies when placed in a Dry Ice-bath at -80° (Note 4).

(d) *Cyclobutane- H_8^2* . Cyclobutene- H_6^2 is hydrogenated with hydrogen- H_2^2 gas to obtain cyclobutane- H_8^2 (Note 5).

B. Notes

1. The following synthesis of cyclobutene- H_6^2 and cyclobutane- H_8^2 is according to the procedure of Cope¹ with modifications.

2. In the procedure of Cope,¹ the cycloöctatetraene was reduced with sodium in a mixture of ether and liquid ammonia. The product was a mixture of 1,3,5-cycloöctatriene, bicyclo[4.2.0]octa-2,4-diene and 1,3,6-cycloöctatriene.

3. 1,3,5-Cycloöctatriene forms a silver nitrate complex, m.p. 125–126°. The complex can be recrystallized from absolute alcohol and is decomposed by adding it to water and then adding concentrated ammonium hydroxide, with stirring, until the precipitate of silver hydroxide disappears. The hydrocarbon, dried by passage through silica gel, can then be distilled rapidly at 0.5 mm. with a pot temperature below 40°, n_D^{25} 1.5249.

4. The cyclobutene prepared by Cope¹ was identified by comparison of its infrared spectrum with the spectrum of an authentic sample prepared from *N,N*-dimethylcyclobutylamine oxide,² by conversion to the dibromide and by oxidation with neutral aqueous sodium permanganate to succinic acid, m.p. 184–185°. The residue from the pyrolysis was shown to be methyl phthalate.

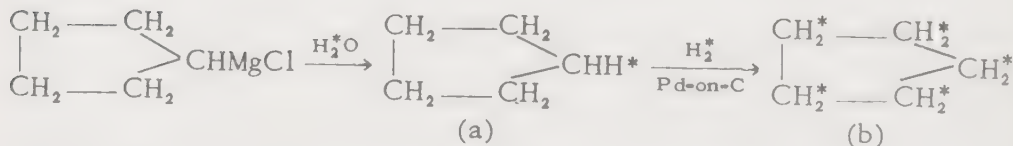
5. According to Lord, the cyclobutene- H_2^2 is conveniently handled by forming dibromocyclobutane- H_2^2 from which it is conveniently regenerated with zinc dust in alcohol.³ The catalyst and conditions for the hydrogenation were not given. Presumably a platinum or palladium catalyst could be used at ordinary temperature and pressure.

¹A. C. Cope, A. C. Haven, Jr., F. L. Ramp and E. R. Trumbull, *J. Am. Chem. Soc.*, 74, 4867 (1952).

²J. D. Roberts and C. W. Sauer, *ibid.*, 74, 3192 (1952).

³G. B. Heisig, *ibid.*, 63, 1698 (1941).

CYCLOPENTANE- H_{10}^2



F. A. Miller and R. G. Inskeep, *J. Chem. Phys.*, 18, 1519 (1950).

A. Procedure

(a) *Cyclopentane- H_1^2* . A solution of cyclopentylmagnesium chloride is prepared by the addition of cyclopentyl chloride to magnesium turnings in dry butyl ether. The theoretical amount of hydrogen- H^2 chloride, prepared from water- H_2^2 and benzoyl chloride,¹ is bubbled slowly into the ether solution under an atmosphere of dry nitrogen. The resulting cyclopentane- H_1^2 is distilled from the mixture and then fractionated.

(b) *Cyclopentane- H_{10}^2* . Cyclopentane- H^2 , is refluxed in the small reservoir of the apparatus (Note 1) so that its vapor is mixed with a stream of

hydrogen- H_2^2 gas. The mixture is passed over the heated palladium-on-carbon catalyst (Note 2), maintained at $190-210^\circ$, and then through a condenser cooled with acetone at Dry Ice-temperature. The condensate is returned to the reservoir through a 1-mm. capillary. The exit hydrogen- H_2^2 is then passed through a cold trap. Eight ml. of cyclopentane- H_{11}^2 , is evaporated at approximately 0.02 mole per minute, and the hydrogen- H_2^2 flow is about 0.001 mole per minute. The exchange is continued for a total of 66 hours, and a yield of 5.5 ml. of cyclopentane- H_{10}^2 is obtained (Note 3).

B. Notes

1. A diagram of the exchange apparatus is given by Miller and Inskeep.
2. Exchange between cyclohexane and deuterium occurs over a catalyst of platinum-black-on-platinum foil.^{2,3} In this work the activity of this catalyst rapidly declined over 12 to 18 hours. It may have been poisoned by silicon stopcock grease.⁴ The palladium catalyst proved quite satisfactory.
3. The product was 96 mole per cent $C_5H_{10}^2$; the chief impurity was cyclopentane- H_9^2 .

¹H. C. Brown and C. Groot, J. Am. Chem. Soc., 64, 2223 (1942).

²C. Horrex, R. K. Greenhalgh and M. Polanyi, Trans. Faraday Soc., 35, 511 (1938).

³A. Farkas and L. Farkas, *ibid.*, 35, 917 (1939).

⁴R. H. Savage, J. Chem. Phys., 16, 237 (1948).

CYCLOHEXANE- H_{12}^2



A. Langseth and B. Bak, J. Chem. Phys., 8, 403 (1940).

A. Procedure

(a) *Cyclohexane- H_{12}^2* . Benzene- H_6^2 is reduced at 180° with hydrogen- H_2^2 in the presence of a nickel catalyst. The equilibrium mixture of benzene- H_6^2 and cyclohexane- H_{12}^2 , thus obtained, is purified by treatment with bromine and a small quantity of iron powder, in the dark. Under these conditions the benzene- H_6^2 is rapidly brominated, and the cyclohexane- H_{12}^2 is scarcely attacked. The product is washed with water, dried and distilled, b.p. $77.8-78.0^\circ$ (760 mm.) (Note 1).

(b) *Cyclohexane- H_{11}^2* . This compound is obtained from cyclohexylmagnesium bromide by hydrolysis with water- H_2^2 . (See benzene- H_1^2 and toluene-2- H^2).

To a solution of 3.0 g. (0.0717 mole) of lithium aluminum hydride-H₂ in 200 ml. of dry ether, contained in a 3-necked flask fitted with a mechanical stirrer, a reflux condenser with calcium chloride tube and a glass stopper, is added 22.2 g. (0.0717 mole) of L-menthyl *p*-toluenesulfonate,¹ in portions (Note 1). The reduction is completed by stirring and gently refluxing this mixture overnight; during this time, a white, granular solid separates. The metal complex and excess hydride are decomposed by the cautious addition of a solution of 11 ml. of water in 40 ml. of dioxane, followed by 100 ml. of water and then enough concentrated hydrochloric acid to dissolve the aluminum salts. The layers are then separated, the aqueous phase is extracted twice with 10-ml. portions of ether, and the combined ether extracts are dried over anhydrous magnesium sulfate. Most of the ether is removed on a water-bath, 100 ml. of petroleum ether is added to the residue, and the resulting solution is washed successively with three 25-ml. portions of water, six 75-ml. portions of concentrated sulfuric acid and four 50-ml. portions of water. The solution,

after drying over anhydrous potassium carbonate, is filtered into a modified Claisen flask, and the petroleum ether is removed on a water-bath. Upon distillation of the residue two fractions (Note 2) are obtained at 55° (17 mm.): 0.3 g., n_D^{20} 1.4365 and 4.6 g. (46%), n_D^{20} 1.4372. The latter fraction has the following additional properties: d_{20}^{20} 0.7971, d_4^{20} 0.7956 (Note 3), α_D^{25} $-0.07 \pm 0.01^\circ$ ($l = 1$, no solvent), α_D^{25} $-0.14 \pm 0.02^\circ$ ($l = 2$, no solvent), $[\alpha]_D^{25}$ $0.09 \pm 0.01^\circ$ ($l = 2$, no solvent) (Notes 3 and 4).

B. Notes

1. Schmid and Karrer² obtained *trans-p*-menthane with lithium aluminum hydride.

2. Both fractions distilled at the same temperature.

3. This value is in agreement with that calculated by the formula of McLean and Adams³ from the density of the hydrogen analog.

4. The infrared curve showed no absorption in the alcohol (3300–3700 cm^{-1}) or olefin (1650 cm^{-1}) regions, but there was strong absorption in the region, 2133 cm^{-1} , which is characteristic of the C—H² bond stretching frequency.

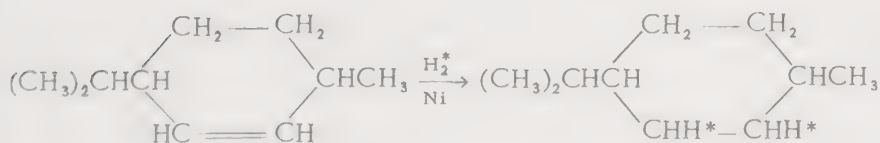
5. Upon treatment of a sample of the *trans-p*-menthane-3-H² in ether with 45 lbs. of hydrogen for 4 hours, over Adams platinum catalyst, no hydrogen was absorbed. The recovered material was apparently unchanged: b.p. 54° (17 mm.), n_D^{20} 1.4371, α_D^{25} $-0.07 \pm 0.01^\circ$ ($l = 1$, no solvent). The infrared absorption curve was unchanged, also.

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trans-p-MENTHANE-2,3-H²



E. R. Alexander and A. G. Pinkus, J. Am. Chem. Soc., 71, 1786 (1949).

A. Procedure

trans-2-*p*-Menthene (Note 1), 6.38 g. (0.0461 mole), is dissolved in 10 ml. of purified diethylcarbitol and reduced with hydrogen-H² at two atmospheres pressure using a Raney nickel catalyst (Note 2). After two hours, 0.0222 mole (96%) of deuterium is absorbed (Note 3). The solution is filtered; the filtrate is added to 400 ml. of water in a separatory

funnel, shaken, and extracted several times with petroleum ether. The extracts are combined and washed, in turn, with five 30-ml. portions of water, nine 20-ml. portions of cold, concentrated sulfuric acid, three 30-ml. portions of 5% sodium carbonate solution and five 30-ml. portions of distilled water. The solution is then dried over sodium sulfate and filtered into a modified Claisen flask. The petroleum ether is removed *in vacuo*. Distillation of the residue gives 2.36 g. (35.9%) of product, b.p. 58° (15 mm.) (Notes 4 and 5).

B. Notes

1. *trans*-2-*p*-Menthene is prepared according to the procedure of Hückel and co-workers.^{1,2}

2. The catalyst was washed several times, by decantation, with diethylcarbitol before adding the solution of olefin.

3. The filtered solution had a rotation of $+1.25^{\circ}$ ($l = 2$), corresponding to about 96% reduction. In previous runs, the addition of fresh catalyst and repeated hydrogenation produced no significant change in the optical rotation, either when hydrogen or when hydrogen- H_2^2 was employed. However, in one experiment complete reduction was obtained at 105° and 2000 pounds pressure, as evidenced by the lack of optical activity in the resulting solution.

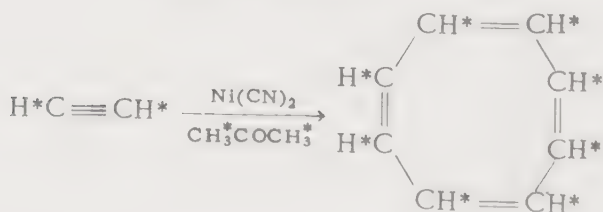
4. Actually the distillate was collected in 3 fractions: 0.21 g. and 2.04 g., both with n_D^{20} 1.4359, and 0.11 g. with n_D^{20} 1.4360. The second fraction, 2.04 g., had in addition: d_{20}^{20} 0.8003, d_4^{20} 0.7989, α_D^{25} $-0.07 \pm 0.01^{\circ}$ ($l = 1$, no solvent), α_D^{25} $-0.14 \pm 0.01^{\circ}$ ($l = 2$, no solvent), $[\alpha]_D^{25}$ -0.09° ($l = 2$, no solvent).

5. Treatment of the product with hydrogen at 2 atmospheres pressure over Raney nickel catalyst did not change any of the physical properties including the rotation, α_D^{25} $-0.07 \pm 0.01^{\circ}$ ($l = 1$, no solvent).

¹W. Hückel and W. Tappe, *Ann.*, 537, 113 (1938).

²W. Hückel, W. Tappe and G. Legutke, *Ann.*, 543, 191 (1940).

CYCLOOCTATETRAENE- H_8^2



A. Procedure

Cyclooctatetraene is prepared¹⁻⁴ by the polymerization of acetylene over a nickel cyanide catalyst (Note 1). Dry acetylene- H_2^2 (99+%), 12-13 g., is prepared from calcium carbide (Note 2) and 25 g. of water- H_2^2 and collected in a tube containing 20-25 ml. of 2-propanone- H_6^2 (Note 3) at Dry Ice-temperature. The solution is quickly poured into a 200-ml. reaction bomb, precooled with Dry Ice, 0.1 g. of catalyst (Note 4) is added, and the bomb is closed. As the bomb warms slightly, enough acetylene- H_2^2 is released to sweep out the enclosed air. The bomb is then heated at 90° for 12 hours (Note 5). Then, the cyclooctatetraene- H_8^2 , 2-propanone- H_6^2 and the by-products are distilled from the bomb under vacuum, and the low-boiling components are separated by fractionation. The product is then purified by fractional recrystallization (Note 6), and the final product has the following physical properties: b.p. $140-143^\circ$; f.p. -4.5° ; n_D^{25} 1.522.

B. Notes

1. The preparation of this catalyst is described by Reppe.¹
2. The calcium carbide was freshly heated at 250° .
3. According to Reppe,¹ tetrahydrofuran is superior to benzene or acetone as polymerization solvent. In this case, it was considered desirable to use an H_2^2 -solvent because of the possibility of hydrogen exchange between the solvent and the acetylene- H_2^2 . 2-Propanone- H_6^2 was used because of the difficulty of making tetrahydrofuran- H_8^2 .
4. The catalyst used by Reppe¹ was a mixture of nickel cyanide and calcium carbide in a ratio of 2:5. Cope and Estes² used nickel acetylacetonate and calcium carbide, 1:2.
5. These conditions are from the work of Cope and Estes.²
6. In addition to about 20% benzene- H_6^2 , the product contained 3-7% styrene- H_8^2 . Styrene-free cyclooctatetraene can be obtained readily by making use of the cyclooctatetraene-silver nitrate complex.¹ The marked difference in the freezing points of the isomeric styrene and cyclooctatetraene (-31° and -4.7° , respectively) makes possible their separation by fractional crystallization.

¹W. Reppe, O. Schlichting, K. Klager and T. Toepel, *Ann.*, 560, 1 (1948).

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⁴J. W. Copenhaver and M. H. Bigelow, *Acetylene and Carbon Monoxide Chemistry*, Reinhold, New York, 1949.

METHANE- H_1^2 

W. H. J. Childs and H. A. Jahn, Proc. Roy Soc. (London), 169A, 428 (1939).

A. Procedure (Note 1)

In a 1-l. flask, equipped with a reflux condenser and dropping funnel and provided with an inert atmosphere, is placed 24.5 g. of magnesium turnings. To start the reaction, about 35 ml. of a solution of 62 ml. of methyl iodide in a like amount of amyl ether is added (Note 2). Then the remainder of the amyl ether, 345 ml., is added slowly in 2 or 3 portions. When the first reaction is apparently complete, the remaining methyl iodide is added in portions.

The flask containing the Grignard reagent is attached *via* a flexible metal joint to a vertical, spiral water-condenser and thence to a vacuum manifold made up of a spiral condenser, a cold trap and storage bulbs in series (Note 3). To the amyl ether solution of methylmagnesium iodide is then added an equal volume of dioxane (Note 4), with thorough mixing. This mixture is frozen, and the apparatus is evacuated. The two spiral condensers are cooled with water and alcohol-Dry Ice, respectively. To the melted reaction mixture then is added slowly, from the dropping funnel, a solution of water- H_2^2 in a 10-fold volume of dioxane. As the mixture is agitated, the evolved gas is allowed to collect in the reaction flask until a manometer on the line indicates a pressure near atmospheric. Then a stopcock preceding the cold trap is opened, and the gas is liquified in the trap with liquid air. This process is repeated as long as gas is evolved. The combined product is vacuum-distilled into a second trap and finally collected in a storage bulb. The yield of methane- H_1^2 , density 0.762 ± 0.001 g./l. at S. T. P., is 72% (Note 5).

B. Notes

1. A total of 40 l. at S. T. P. of methane- H_1^2 was desired, so the methylmagnesium iodide reagent was prepared in three equal amounts. The preparation of one portion is described.

2. Ethyl ether was replaced by the less volatile amyl ether to simplify purification.

3. A diagram of the apparatus is shown by Childs and Jahn.

4. When water was added directly to the ethereal solution of Grignard reagent, the yield of methane was only 20%. It was suspected that the lumpy precipitate of magnesium hydroxide and magnesium iodide trapped much of the water; stirring and heating the mixture did not improve the yield. Dry, purified dioxane was added as an inert diluent to slow the reaction and disperse the precipitate. When dioxane was added, in ex-

cess, to the ether solution, a precipitate of the Grignard reagent is formed, which with agitation forms a smooth cream. When water diluted with dioxane is added to this mixture, there is a steady evolution of methane, and the mixture retains its creamy consistency. With small quantities of materials, the yield of methane was 93%; heating of the reagents was unnecessary.

5. The infrared spectrum of the product indicated the absence of ordinary methane.

C. Other Preparations

Methane- H_1^2 has been obtained by the action of water- H_2^2 on methylmagnesium iodide;¹⁻⁷ by the reaction of methyl iodide and water- H_2^2 on a mercury-aluminum couple;⁸ by the reaction of methyl radicals with hydrogen- H_2^2 ;⁹⁻¹³ by the photolysis of acetone in hydrogen- H_2^2 ;¹⁴ by thermal decomposition of ethane- H_2^2 ;¹⁵ and by the reaction of acetyl peroxide and 1-chloro-2-methylpropane-1- H_1^2 .¹⁶ Wagner and Stevenson² reported a 94% yield.

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METHANE- H_2^2



V. H. Dibeler and F. L. Mohler, J. Research Nat. Bur. Standards, 45, 441 (1950).

A. Procedure

Methane- H_2^2 is prepared according to an adaptation¹ of the method of Finholt.² A 250-ml. Claisen-type flask is connected to a condenser by

means of a standard tapered joint (Note 1). The condenser is provided with connections to a vacuum system, an open-end manometer, a cold trap and a Toepler pump (Note 2). Approximately 0.5 g. (13 mmoles) of powdered lithium aluminum hydride- H_4^2 (Note 3) and 50 ml. of anhydrous butyl ether are introduced into the reaction flask. A break-seal tube containing 1.7 g. (10 mmoles) of methylene bromide, *in vacuo*, is attached to the flask which is cooled to 0° and cautiously evacuated to degas the mixture. The flask, condenser and manometer are isolated from the rest of the system, and the flask is slowly warmed to 60° and held at that temperature for about 3 hours (Note 4). The solution is cooled with liquid nitrogen, and the system is evacuated. Then the break-seal is opened, and the halide is distilled into the reaction flask. The reagents are allowed to warm slowly until a moderate reaction occurs at about the melting point of the halide (Note 5). When no further rise in pressure is indicated on the manometer, the flask is surrounded with an ice-bath at 0° . From pressure readings at the two temperatures the yield of methane- H_2^2 (93%) is calculated (Note 6).

B. Notes

1. A diagram of the apparatus is given by Dibeler.¹
2. The pump was used to fill sample bulbs with the reaction products.
3. Lithium aluminum hydride- H_4^2 is available from Metal Hydrides Inc., Beverly, Mass. The hydride is powdered under an atmosphere of dry nitrogen.
4. Under these conditions, gentle refluxing occurs sufficient to stir the mixture.
5. If necessary, the flask is intermittently cooled to maintain moderate reaction.
6. Mass spectrometric analysis of the product indicated a 93% yield of methane- H_2^2 , with a ratio of methane- H_2^2 to hydrogen- H_2^2 (mole % ratio) of 13.

C. Other Preparations

Methane- H_2^2 has been prepared by the reaction of methylene iodide and water- H_2^2 with an aluminum-mercury couple.³ Methane- H_2^2 has also been obtained by the photolysis of acetone in the presence of hydrogen- H_2^2 ,⁴ and by the decomposition of methane-methane- H_4^2 mixtures in an electrical discharge.⁵

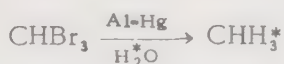
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METHANE- H_3^2 

G. E. MacWood and H. C. Urey, *J. Chem. Phys.*, **4**, 402 (1936).

A. Procedure

An aluminum-mercury couple is prepared by the action of 0.5% mercuric chloride solution on clean aluminum clippings. The couple is washed with 95% alcohol and then dried under vacuum to remove traces of hydrogen compounds, principally water. Bromoform and water- H_2^2 are then distilled onto the couple under vacuum. The reaction proceeds as soon as the mixture reaches room temperature. The methane- H_3^2 is passed through a Dry Ice-trap in order to remove water- H_2^2 vapor and any other condensable material and is stored finally in a glass bulb (Note 1).

B. Notes

1. A poor feature of this method is the reaction of water- H_2^2 with the couple, resulting in a deuterium impurity of about 10%. The simplicity of the method makes it of considerable interest. This may be the method of choice with regard to purity of product since the reduction of bromoform with lithium aluminum hydride- H_4^2 ¹ (see methane- H_2^2) gave a product which was only 60% methane- H_3^2 .

C. Other Preparations

Methane- H_3^2 has been prepared² by the reaction of water with methylmagnesium- H_3^2 bromide or methylmagnesium- H_3^2 iodide; the product contained about 70% methane- H_3^2 , and the remainder was mainly methane- H_2^2 .

Methane- H_3^2 has also been prepared by reduction of bromoform with lithium aluminum hydride- H_4^2 ,¹ by pyrolysis of sodium acetate- H_3^2 with soda lime at 800° under vacuum;³ and by the action of bromoform and water- H_2^2 on an aluminum-mercury couple.⁴ The first of these methods gave a product of about 60% purity mixed with methane- H_4^2 and methane- H_2^2 ; the second method gave a product of about 50% purity with approximately equal amounts of methane- H_2^2 and methane- H_4^2 present as impurities.

Methane- H_3^2 has also been obtained by the reaction of chloroform, ethanol- H^2 and zinc;⁵ by the photolysis of acetone- H_6^2 in the presence of hydrogen;⁶ and by the pyrolysis of acetone- H_6^2 .^{7,8}

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METHANE-H₄²



G. E. MacWood and H. C. Urey, *J. Chem. Phys.*, 4, 402 (1936).

A. Procedure

Methane-H₄² is prepared by the catalytic reduction of carbon dioxide (Note 1). Pure hydrogen-H₂² and carbon dioxide¹ (Note 2), in a ratio of 4:1, are passed over a supported nickel catalyst at 310°. The water formed in the reduction is removed from the product by passage through a trap cooled with solid carbon dioxide. The methane-H₄² is collected as a liquid in a trap cooled with liquid air. When the reaction is complete the product is allowed to vaporize, from a bath at -80°, into a large evacuated storage flask.

B. Notes

1. An excellent kinetic study of the hydrogenation of carbon dioxide and carbon monoxide over nickel was made by Nicolai.²

2. Commercial solid carbon dioxide was used; the gas was purified by passage through 95% sulfuric acid.

C. Other Preparations

Methane-H₄² has been prepared by the reduction of carbon monoxide in the presence of nickel;^{2,3,10} by the reduction of carbon dioxide over nickel;^{4,5} by decomposition of aluminum carbide^{6-9,13} with water-H₂²; and by the reaction of carbon tetrachloride, ethanol-H² and zinc.¹⁰

Methane-H₄² has also been obtained by the photolysis of acetone-H₆² in the presence of hydrogen-H₂² and by pyrolysis¹² of acetone-H₆².

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ETHANE-H₁²



J. Turkevich, L. Friedman, E. Solomon and F. M. Wrightson, J. Am. Chem. Soc., 70, 2638 (1948).

A. Procedure

(a) *Ethane-H₁²*. In a 250-ml. flask, fitted with a vertical condenser protected from moisture by a calcium chloride tube, 11 g. of ethyl bromide is added to 10 ml. of dry butyl ether and 2.8 g. of magnesium turnings. The mixture is gently warmed to initiate the reaction. When the reaction is complete, the flask is attached to a vacuum line, then is cooled in Dry Ice and evacuated. The reagent is thoroughly outgassed by repeated melting, freezing and evacuation, and is then decomposed by the addition of successive portions of water-H₂². Mass spectrometric analysis of the gas samples indicated a constant ratio of abundances of masses 30 and 31 after the first sample.

(b) *Propane-1-H₁²*. In accordance with the above procedure, propylmagnesium bromide is treated with water-H₂².

(c) *Propane-2-H₁²*. The use of isopropylmagnesium bromide in the above procedure affords propane-2-H₁².

B. Other Preparations

Ethane-H₁² has been prepared in 67% yield by the action of water-H₂² on ethyl Grignard reagents^{1,2} and by the hydrolysis of ethylmagnesium-2-H₁² bromide with ordinary water.³ Ethane-H₁² has also been obtained⁴ by the reaction of ethyl iodide with zinc and acetic acid-H₂²; the photolysis⁵ of diethyl ketone in the presence of hydrogen-H₂²; and the reaction of diethylzinc with water-H₂².⁶ The latter reaction is quantitative on a small scale.

Propane-2- H_1^2 has been obtained from isopropyl iodide, zinc and acetic acid- H^2 ;⁴ in 67% yield, by the action of water- H_2^2 on isopropylmagnesium bromide;¹ and from isopropylmagnesium chloride and water- H_2^2 .⁷

Propane-1- H_1^2 has been obtained, in 75% yield, by hydrolysis of the corresponding Grignard reagent¹ and by the reaction of propylmagnesium chloride and water- H_2^2 .⁷

In a study of the reaction of propene and propene- H_6^2 with hydrogen- H_2^2 over a 5% platinum-pumice catalyst, Bond and Turkevich⁶ obtained mixtures of propanes containing from 0 to 8 atoms of deuterium per molecule.

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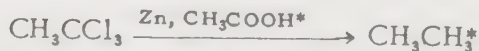
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ETHANE-1- H_3^2



D. O. Schissler, S. O. Thompson and J. Turkevich, Discussions Faraday Soc., 10, 46 (1951).

A. Procedure (Note 1)

(a) *Ethane-1- H_3^2* . The reaction is carried out in a vacuum apparatus consisting of a reactor tube fitted with three side tubes for the storage of the organic halide, the heavy water and the zinc. An excess of redistilled acetic anhydride is placed in the reactor tube, and some thoroughly out-gassed, granulated zinc is added (Note 2). The alkyl halide is then distilled into the reactor at liquid-nitrogen temperature. The mixture is warmed to room temperature (Note 3), and a small portion of deaerated water- H_2^2 (99.8%) is distilled into the reactor. The reaction is allowed to proceed to exhaustion of the acetic acid- H^2 formed, and the product is collected in a sample bulb. Successive small portions of water- H_2^2 are added to the reaction mixture, and the ethane-1- H_3^2 evolved, after each addition, is collected as a separate sample (Note 4). After the third addition of water, the isotopic purity of the product is usually greater than 95% (Note 5).

(b) *Ethane-1- H_2^2* . This compound is prepared in the same manner from 1,1-dibromoethane.

(c) *Propane-2-H₂²*. The use of 2,2-dibromopropane in the above procedure affords propane-2-H₂². (Note 6).

B. Notes

1. This is a general method for the preparation of H²-alkanes. A controllable reaction with a minimum of side reactions is obtained by the use of monoiodides, dibromides and trichlorides.

2. Any evolved hydrogen gas is pumped off.

3. Any hydrocarbon, produced by the action of moisture in the system, is pumped off.

4. In this way, the majority of protium contamination in the system is removed. The mass spectra of successive samples show increasing deuterium content which reaches a constant value.

5. No measurable quantity of hydrogen-H₂² or olefins is produced by this reaction.

6. According to Condon and co-workers,¹ who first reported the preparation of propane-2-H₂², this compound boils at -44° (748 mm.) and has a vapor pressure of 903 mm. at the temperature of melting mercury.

C. Other Preparations

Ethane-1-H₃² has been prepared from 1,1,1-trichloroethane similarly to the procedure described and by the reaction of iodomethane-H₃² with methylmagnesium iodide.² It has also been obtained by photolysis³ of acetone-H₆².

Propane-2-H₂² has been prepared¹ from 2-chloropropane-2-H² via the Grignard reaction with water-H₂².

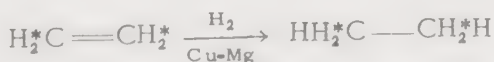
2-Methylpropane-2-H² has been obtained by the same procedure from *t*-butylmagnesium chloride.¹

¹F. E. Condon, H. L. McMurry and V. Thornton, J. Chem. Phys., 19, 1010 (1951).

²R. van Riet, Bull. classe sci., Acad. Roy. Belg., 41, 188 (1955).

³S. W. Benson and C. W. Falterman, J. Chem. Phys., 20, 201 (1952).

ETHANE-1,1,2,2-H₄²



G. Joris, H. S. Taylor and J. C. Jungers, J. Am. Chem. Soc., 60, 1982 (1938).

A. Procedure

(a) *Ethane-1,1,2,2-H₄²*. The hydrogenation of ethylene-H₄² in the presence of a copper-magnesium catalyst (Note 1) is quite rapid at 40°. A

gas mixture is prepared of 2 moles of hydrogen- H_2^2 and 1 mole of ethylene- H_4^2 and transferred into the evacuated catalyst chamber (Note 2) with a Toepler pump. The gas mixture is passed through a trap cooled with a Dry Ice-acetone mixture to minimize access of mercury vapor to the catalyst system. When hydrogenation is complete, as indicated by the change in pressure at constant volume, the ethane- H_4^2 is collected in a liquid nitrogen-cooled trap, and the excess hydrogen- H_2^2 is recovered (Note 3).

(b) *Ethane-1,2- H_2^2* . By the hydrogenation of ethylene with hydrogen- H_2^2 , according to the above procedure, ethane-1,2- H_2^2 is obtained.

B. Notes

1. The copper catalyst¹ is prepared by dehydration and reduction of a co-precipitated mixture of magnesium hydroxide-copper hydroxide in the molecular ratio 4:1.

2. In this comparative study of the rates of hydrogenation of ethylene and ethylene- H_4^2 with hydrogen and hydrogen- H_2^2 , the catalyst was placed on platforms in thin layers.

3. The hydrogenation of 5 cm. of ethylene- H_4^2 with 5 cm. of hydrogen was practically complete in 9 minutes at 40°. Apparently ethylene- H_4^2 reacts with hydrogen more rapidly than does ethylene under these conditions. The composition of the ethane- H_4^2 prepared by catalytic hydrogenation is uncertain since the catalytic exchange of the hydrogens in ethylene is well known.^{2,3}

C. Other Preparations

Ethane-1,1,2,2- H_4^2 has been prepared by the reaction of acetylene and hydrogen- H_2^2 on platinum.⁴

Ethane-1,2- H_2^2 has been prepared by the reaction of ethylene and hydrogen- H_2^2 on a platinum catalyst,² and iron catalyst⁵ and a nickel catalyst.^{6,7}

¹H. S. Taylor and G. Joris, *Bull. soc. chim. Belg.*, 46, 241 (1937).

²A. Farkas and L. Farkas, *J. Am. Chem. Soc.*, 60, 22 (1938).

³A. Farkas, L. Farkas and E. K. Rideal, *Proc. Roy. Soc. (London)*, A146, 630 (1934).

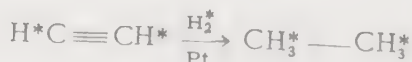
⁴A. Farkas and L. Farkas, *J. Am. Chem. Soc.*, 61, 3396 (1939).

⁵R. Klar, *Z. physik. Chem.*, B27, 319 (1934); through *Chem. Abstracts*, 29, 1383 (1935).

⁶E. K. Rideal, *J. Inst. Petroleum Tech.*, 24, 221 (1938); through *Chem. Abstracts*, 32, 6536 (1938).

⁷G. H. Twigg, *Discussions Faraday Soc.*, 8, 152 (1950).

ETHANE- H_6^2



F. Stitt, *J. Chem. Phys.*, 7, 297 (1939).

A. Procedure

(a) *Ethane-H₆²*. Dry hydrogen-H₂², free of oxygen, is passed over solid acetylene-H₂² in a U-tube trap. The temperature of the latter is so controlled as to give a resulting mixture of hydrogen-H₂² and acetylene-H₂² in the ratio 10:1 (Note 1). This mixture is passed over a platinum-on-asbestos catalyst (Note 2) maintained at about 100°. The ethane-H₆² is removed from the gas stream by passage through a trap cooled with liquid air. The excess hydrogen-H₂², issuing from a small nozzle, is mixed with dry air and burned to water-H₂² which is recovered (Note 3). The product is purified by distillation from a bath at -135° (Note 4).

(b) *Ethane-H₅²*. Acetylene-H₁² is hydrogenated in the same manner to obtain ethane-H₅² (Note 5).

B. Notes

1. Such a high ratio of deuterium to acetylene is not necessary for complete hydrogenation but tends to eliminate polymerized products.

2. The platinum catalyst is prepared by saturating asbestos with platinum chloride solution, followed by drying below 200° in a slow stream of nitrogen and then in a vacuum. Prior to use the dry catalyst is treated with hydrogen-H₂².

3. Oxidation of the excess deuterium was catalyzed by a red hot platinum spiral. Preceding the nozzle was a sintered glass plate which prevented striking back in case an explosive mixture was present.

4. About 6 l. of ethane-H₆² was prepared in this manner. Mass spectrometric analysis of the product indicated an ethane-H₅² content of 2.9%.

5. Actually the acetylene-H₁² prepared from sodium acetylide and water-H₂² contained approximately 47.3% acetylene-H₂². Therefore, the ethane was a mixture of C₂H₆² and C₂HH₅²; in addition, some exchange occurred and an appreciable quantity of C₂H₂H₄² was also present.

C. Other Preparations

Ethane-H₆² has been prepared by the hydrogenation of acetylene-H₂² with hydrogen-H₂² and a copper catalyst,^{1,2} and with a nickel-asbestos catalyst.³ Mass spectrometric analysis, in the latter case, indicated the ethane component to be greater than 99% C₂H₆², together with 3% deuterated propane and butane. Ethane-H₆² has also been prepared from ethylene by deuteration at 0° over a nickel catalyst followed by repeated exchange at 138°,⁴ and by photolysis of acetone-H₆².⁵

Ethane-H₅² has been prepared from ethylene-H₄² via the Grignard reaction.⁶

Reduction⁷ with deuterium of ethyleneplatinous chloride, [bisethylene-dichloro- μ -dichlorodiplatinum(II)],⁸ in toluene solution at -23.7° resulted in the formation of a mixture of compounds including ethane, ethane-H₁²,

H_2^2 -ethane, H_3^2 -ethane, H_4^2 -ethane, ethane- H_5^2 , ethane- H_6^2 , ethylene, ethylene- H_1^2 , ethylene-1- H_2^2 , ethylene-1,2- H_2^2 , ethylene- H_3^2 and ethylene- H_4^2 . The reduction⁹ of solid ethyleneplatinous chloride with deuterium at -22.0° resulted in the formation of a mixture of all the ethanes from C_2H_6 to $C_2H_6^2$.

¹R. N. Pease and A. Wheeler, J. Am. Chem. Soc., 57, 1144 (1935).

²G. Joris, H. S. Taylor and J. C. Jungers, *ibid.*, 60, 1982 (1938).

³L. A. Wall and W. J. Moore, *ibid.*, 73, 2840 (1951).

⁴K. Morikawa, W. S. Benedict and H. S. Taylor, *ibid.*, 58, 1796 (1936).

⁵S. W. Benson and C. W. Falterman, J. Chem. Phys., 20, 201 (1952).

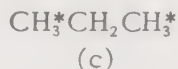
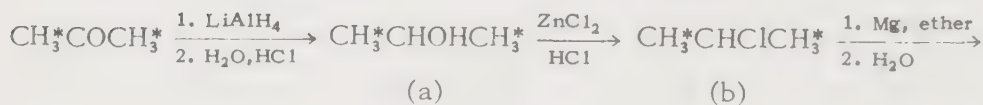
⁶R. van Riet and M. de Hemptinne, Ann. soc. sci. Bruxelles, Ser. I, 64, 177 (1950); through Chem. Abstracts, 45, 10055 (1951).

⁷J. H. Flynn and H. M. Hulburt, J. Am. Chem. Soc., 76, 3393 (1954).

⁸J. S. Anderson, J. Chem. Soc., 1934, 971.

⁹J. H. Flynn and H. M. Hulburt, J. Am. Chem. Soc., 76, 3396 (1954).

PROPANE-1,3- H_6^2



F. E. Condon, J. Am. Chem. Soc., 73, 4675 (1951).

A. Procedure

(a) *2-Propanol-1,3- H_6^2* . Acetone- H_6^2 (0.2 mole) is added to 0.1 mole of lithium aluminum hydride in 50 ml. of ether.¹ When the reaction is complete, 75 ml. of water containing about 0.4 mole of hydrochloric acid is added dropwise. The ether layer is dried with Drierite and fractionated (Note 1). The water layer is also fractionated, and the azeotrope² (b.p. 80.38°) is collected (Note 2). The total yield is quantitative.

(b) *2-Chloropropane-1,3- H_6^2* . To 0.2 mole of 2-propanol-1,3- H_6^2 is added 0.7 mole of concentrated hydrochloric acid and 1 mole of zinc chloride. The 2-chloropropane-1,3- H_6^2 is slowly distilled from the reaction mixture, collected in an ice-cooled receiver, washed with ice-water, dried with anhydrous potassium carbonate and fractionated (Note 1). Material of boiling range $33-35^\circ$ is obtained in 70% yield; n_D^{20} 1.3744; d_4^{20} 0.9247.

(c) *Propane-1,3- H_6^2* . A Grignard reagent is prepared from 0.13 mole of 2-chloropropane-1,3- H_6^2 , 0.13 g. atom of magnesium and 150 ml. of ether. After preparation of the reagent, the solution is warmed under reflux for about 1 hour to eliminate by-product hydrocarbons (Note 3). Excess water is added slowly, and the generated propane-1,3- H_6^2 , together with consid-

erable ether, is collected in a Dry Ice-cooled trap. The material collected is fractionated in a Podbielniak Heligrid low-temperature column. The C_3 fraction obtained is redistilled through the column. The propane-1,3- H_6^2 , obtained in 70% yield, distills from -46.0° (750 mm.) to -51° (560 mm.).

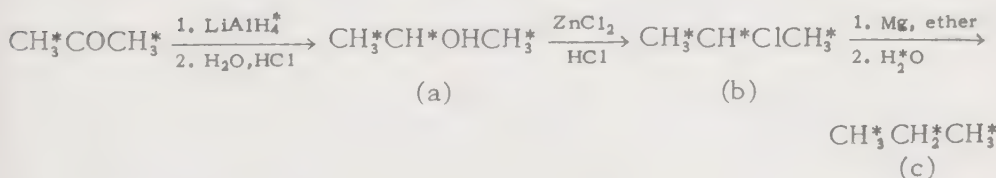
B. Notes

1. A 30×0.7 -cm. column with Heligrid packing was used.
2. The azeotropic product was found to contain 10.45% water by the Karl Fischer method.
3. The hydrocarbons eliminated were collected in a cold trap and fractionated in order to obtain a C_3 fraction. Of this, about 60% proved to be H^2 -propane homologs.

¹R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 1197 (1947).

²L. H. Horsley, Ind. Eng. Chem., Anal. Ed., 19, 508 (1947).

PROPANE- H_3^2



F. E. Condon, J. Am. Chem. Soc., 73, 4675 (1951).

A. Procedure

(a) 2-Propanol-1,2,3- H_7^2 . To a solution of 0.1 mole of lithium aluminum hydride- H_4^2 (Note 1) in 75 ml. of absolute ether is added a solution of 0.3 mole of acetone- H_6^2 in ether.¹ The reaction mixture is treated cautiously with 75 ml. of water containing 0.4 mole of hydrochloric acid. The ether layer is separated, dried over Drierite and fractionated (Note 2). The water layer is also fractionated, and the 2-propanol-1,2,3- H_7^2 is collected as the azeotrope (b.p. 80.38°) (Note 3). The total yield of 2-propanol-1,2,3- H_7^2 is quantitative.

(b) 2-Chloropropane- H_7^2 . To 1 mole of hydrochloric acid and 1.5 moles of zinc chloride is added 0.3 mole of 2-propanol-1,2,3- H_7^2 . 2-Chloropropane- H_7^2 is slowly distilled from the mixture, collected in an ice-cooled receiver, washed with ice-water, dried with anhydrous potassium carbonate, and distilled (Note 2). The yield is about 70%, b.p. $33-35^\circ$; n_D^{20} 1.3748; d_4^{20} 0.9386.

(c) Propane- H_8^2 . 1-Methylethylmagnesium- H_7^2 chloride is prepared from 0.19 mole of 2-chloropropane- H_7^2 and 0.19 g. atom of magnesium in 150 ml.

of ether. This reagent is treated with 0.3 mole of water- H_2^2 (99.8%) and then 0.2 mole of water- H_2^2 (99.3%). The propane- H_3^2 , obtained in 75% yield, distills from -46° (750 mm.) to -51° (560 mm.) (Note 4).

B. Notes

1. Obtainable from Metal Hydrides Inc., Beverly, Mass.
2. A 30×0.7 -cm. column with Heligrid packing was used.
3. Analysis by Karl Fischer method indicated 7.11% water in the distillate.
4. The propane- H_3^2 is fractionated in a Podbielniak Heligrid low-temperature column to separate a C_3 fraction from the entrained ether. The C_3 fraction is redistilled.

*R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 1197 (1947).

2-METHYLPROPANE-2- H^2



H. C. Brown and G. A. Russell, J. Am. Chem. Soc., 74, 3995 (1952).

A. Procedure

A solution of 232.5 g. (2.5 moles) of freshly distilled *t*-butyl chloride is dissolved in sufficient anhydrous ether to make a total volume of 1500 ml. During a 10-hour period, this solution is added, with stirring, to 60 g. (2.5 g. atom) of magnesium turnings and 150 ml. of ether in a 2000-ml. 3-necked flask equipped with a mercury-sealed stirrer, a pressure-equalized dropping funnel and a Friedericks condenser fitted with soda-lime and calcium hydride drying tubes. The resulting solution is concentrated to 1500 ml., and an aliquot is analyzed (Note 1). The hydrogen- H^2 chloride, prepared¹ from 20 g. (1 mole) of water- H_2^2 , is passed through an ice-trap and over the surface of the vigorously stirred Grignard reagent. Water at 0° is circulated through the Friedericks condenser, and the gas passing through this condenser is condensed in a Dry Ice-cooled trap.

After addition of the hydrogen- H^2 chloride, the ether solution is refluxed for 1 hour, and during this time approximately 300 ml. of a mixture of 2-methylpropane-2- H^2 and ether is collected in the cold trap. To remove the excess ether, this mixture is distilled through a reflux condenser at 0° . The 225 ml. of material collected is treated with bromine (Note 2) and again distilled through the reflux condenser at 0° . The resulting 2-methylpropane-2- H^2 is carefully rectified in a 40-cm. vacuum-jacketed column packed with glass helices and equipped with a low-temperature head. A fraction of 104.5 g. (1.77 moles) boiling at -12.5 to

-11.8° (743 mm.) is collected. The yield is 88.5% based on water- H_2^2 used in making hydrogen- H^2 chloride.

B. Notes

1. The concentration of Grignard reagent was determined by titration with hydrochloric acid and indicated the yield of *t*-butylmagnesium chloride to be 2.20–2.24 moles (88–90%).

2. Addition of 4 ml. of bromine produced a reddish color which persisted after the solution was refluxed 0.5 hour in the dark.

C. Other Preparations

In a study² of the mechanism of catalytic hydrogenation of olefins, *cis*-2-butene, isobutylene and ethylene were treated with large excesses of hydrogen- H_2^2 over a commercial catalyst of nickel-on-kieselguhr. The product of the reaction with *cis*-2-butene at -78° contained all isotopic species from C_4H_{10} to $C_4H_{10}^2$ in an essentially random distribution of the hydrogen- H^2 atoms. Similar results were obtained with isobutylene and ethylene. These results indicate that catalytic hydrogenation is of limited utility for the preparation of specific isomers of deuterium-labeled paraffin hydrocarbons.

¹H. C. Brown and C. Groot, J. Am. Chem. Soc., 64, 2223 (1942).

²J. N. Wilson, J. W. Otvos, D. P. Stevenson and C. D. Wagner, Ind. Eng. Chem., 45, 1480 (1953).

BUTANE-1- H_1^2



C. D. Hurd and J. L. Azorlosa, J. Am. Chem. Soc., 73, 33 (1951).

A. Procedure

To a vigorously stirred Grignard reagent, prepared from 24 g. of magnesium, 100 g. of 1-bromobutane and 400 ml. of butyl ether, is added slowly 11.0 g. of water- H_2^2 . The solution is then heated, and the liberated butane-1- H_1^2 is collected in a trap at -78° (Note 1). The collected butane-1- H_1^2 is purified by distillation through a Davis column; acetone and Dry Ice are used to chill the still-head reservoir to about -10° (Note 2). The yield of material, b.p. -0.5 to -0.2° (740 mm.), is 27.6 g. (85.2%).

B. Notes

1. Preliminary experiments with ordinary water showed that it was necessary to reflux the butyl ether to expel all the butane, and yields

were lowered 10-15% if stirring was not maintained throughout the heating period.

2. At this temperature the reflux ratio is about 5 to 1.

C. Other Preparations

Wagner and Stevenson¹ have also prepared butane-1-H₁², by the method described, in 82% yield, and used a special method of mass spectrometric analysis,² involving low voltage electrons, in determining ordinary alkanes, as well as olefins, present as impurities.

In a study of deuterium isotope effect, Wiberg³ has prepared butane-1-H₁² by the reaction of both butylmagnesium bromide and butyllithium with methanol-H². The isotope effects, k_H/k_{H^2} , in the two instances were, respectively, 0.83-0.87 and 0.99-1.00.

¹C. D. Wagner and D. P. Stevenson, J. Am. Chem. Soc., 72, 5785 (1950).

²D. P. Stevenson and C. D. Wagner, *ibid.*, 72, 5612 (1950).

³K. B. Wiberg, *ibid.*, 77, 5987 (1955).

BUTANE-2,3-H₄²



J. R. McNesby, C. M. Drew and A. S. Gordon, J. Phys. Chem., 59, 988 (1955).

A. Procedure

3-Pentanone-2,4-H₄² is photolyzed in a water-cooled 200-ml. fused silica reaction vessel at 33 mm. initial pressure and at 30°, using a Hanovia Alpine burner. The photolysis is continued for about 2 hours or until the pressure has risen to 45 mm. The entire content of the photolysis vessel is then pumped into a 2-l. flask. This process is repeated 35 times (Note 1).

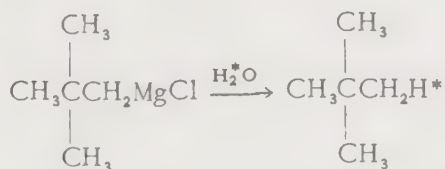
The ethane-1-H₂²/₁-1-H₃²/₁, ethylene-1-H₂²/₁-H₃²/₄ and butane-2,3-H₄² (Note 2) are separated quantitatively by means of fractional desorption from silica gel using nitrogen as the eluant. The separation is monitored by means of a matched pair of thermal conductivity cells, one of which has pure nitrogen flowing through it at about 40 ml. per minute, while the other contains the desorbed gas as well as nitrogen. When the recorder indicates that a hydrocarbon is being desorbed, the gas stream is directed to a refrigerated trap. After pumping most of the nitrogen from the refrigerated butane-2,3-H₄² trap, the butane fraction is evaporated into a 400-ml. flask equipped with a mercury cutoff valve. After the product is freed of residual nitrogen by successive freezing and melting under vacuum, it has a pressure of 60 mm. at room temperature.

B. Notes

1. According to the work of Kutschke,¹ the main products of the photolysis, near room temperature, are butane, ethylene, ethane and carbon monoxide.

2. Mass spectrometric analysis indicated that the ethane was 96.5% $\text{CH}_3\text{CHH}_2^2$ and 3.4% CH_3CH_3^2 . The ethylene was principally $\text{CH}_2=\text{CH}_2^2$ with 0.5% $\text{CHH}^2=\text{CH}_2^2$. There was no H_5^2 -butane in the product, but, based on the purity of the water- H_2^2 used in preparation of the 3-pentanone-2,4- H_4^2 , there may have been 1-2% of butane-2,3- H_3^2 .

¹K. O. Kutschke, M. H. J. Wijnen and E. W. R. Steacie, J. Am. Chem. Soc., 74, 714 (1952).

2,2-DIMETHYLPROPANE- H_1^2 

F. C. Whitmore, G. H. Fleming, D. H. Rank, E. R. Bordner and K. D. Larson, J. Am. Chem. Soc., 56, 749 (1934).

A. Procedure

2,2-Dimethylpropylmagnesium chloride is prepared from 1-chloro-2,2-dimethylpropane according to the procedure of Whitmore and Fleming¹ (Note 1). This material is then reacted with 2.5 ml. of water- H_2^2 to obtain 6.9 g. (78%) of 2,2-dimethylpropane- H_1^2 , m.p. -22 to -21° ; $n_D^{25} 1.35360$ (Note 2).

B. Notes

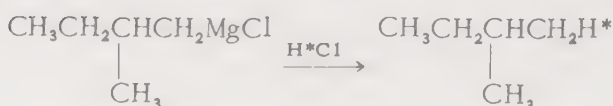
1. 1-Chloro-2,2-dimethylpropane was prepared by direct chlorination of 2,2-dimethylpropane, but could not be prepared from the alcohol and dry hydrogen chloride.² The chloride reacts with magnesium slowly in dilute ether solution.

2. Rank³ reported the preparation of 2,2-dimethylpropane- H_1^2 and tetramethylsilane- H_1^2 , of approximately 99% purity, also *via* the Grignard reaction.

¹F. C. Whitmore and G. H. Fleming, J. Am. Chem. Soc., 55, 4161 (1937).

²F. C. Whitmore and H. S. Rothrock, *ibid.*, 54, 3431 (1932).

³D. H. Rank, B. D. Saksena and E. R. Shull, Discussions Faraday Soc., 9, 187 (1950).

2-METHYLBUTANE-1-H²

H. C. Brown and C. Groot, J. Am. Chem. Soc., 64, 2563 (1942).

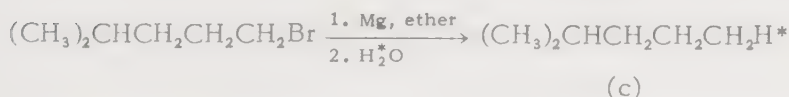
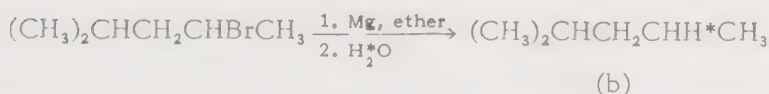
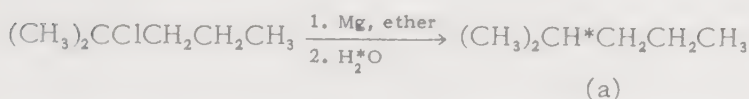
A. Procedure

A 2-l. 3-necked flask is fitted with a 500-ml. dropping funnel, a mercury-sealed stirrer and a reflux condenser protected by a calcium chloride tube. In the flask are placed 36 g. (1.5 moles) of magnesium, 100 ml. of butyl ether and a few drops of methyl iodide, as catalyst. 1-Chloro-2-methylbutane, 143 g. (1.33 moles), is placed in the funnel, a few ml. is run into the flask, and the mixture is warmed on the steam-bath to start the reaction. The halide is then mixed with about 350 ml. of butyl ether, in the funnel, and added to the reaction mixture dropwise (Note 1) during 2 hours. After the reaction mixture stands one hour, heat is no longer evolved, and the reaction is assumed to be complete. The mixture is then diluted to a total volume of 1 l. with butyl ether. The yield of Grignard reagent (Note 2) is 1.16 moles (87%). The apparatus for generating hydrogen-H² chloride¹ is connected to the flask in place of the dropping funnel, and the hydrogen-H² chloride from 11.7 g. (1.17 equiv.) of water-H₂ is passed into the solution during 2 to 3 hours. The solution is cooled with an ice-bath and agitated vigorously to prevent caking of the magnesium salt. When the reaction is complete, the flask is equipped with a stillhead and a condenser which is connected to the receiver by an adaptor with side-arm. A Dry Ice-cooled trap is attached to the side arm. With the receiver cooled in ice, the flask is heated in an oil-bath at about 150° until the inside temperature is 142° (b.p. of butyl ether). The crude product, which weighs 79 g. (92%), is fractionated in a Podbielniak Heligrid column (Note 3).

B. Notes

1. The reaction is controlled by cooling the flask with a water-bath at 20–25°.
2. A 2-ml. sample was added to about 40 ml. of 0.1 N acid, which was heated to boiling and back titrated with standard base, using phenolphthalein indicator.
3. The column was operated with solid carbon dioxide as refrigerant in the partial takeoff distilling head.

¹H. C. Brown and C. Groot, J. Am. Chem. Soc., 64, 2443 (1942).

2-METHYLPENTANE-5-H₁²

D. P. Stevenson, C. D. Wagner, O. Beeck and J. W. Otvos, J. Am. Chem. Soc., 74, 3269 (1952).

A. Procedure

(a) *2-Methylpentane-2-H²*. Under an atmosphere of nitrogen in a 3-necked flask equipped with a mercury-sealed stirrer, a reflux condenser and a dropping funnel, a solution of 12.8 ml. of 2-chloro-2-methylpentane (Note 1) in 40 ml. of dry ether is added slowly to 2.6 g. of magnesium turnings. The dropping funnel is replaced by a rubber policeman, and the mercury-sealed stirrer is replaced by a stainless steel rod inserted through a rubber stopper. With the reaction mixture cooled in ice, 6.5 ml. of water-H₂² is added in portions as a fine spray from a hypodermic needle which is inserted through the rubber policeman. The mixture is stirred with the steel rod to break up aggregates of basic magnesium salt. After the mixture is kept at room temperature for 2 hours, volatile compounds are distilled under vacuum into a cold trap. The distillate is shaken with Ascarite and fractionated in a Podbielniak-type column. The fraction of boiling range 58–70° amounts to 6.8 g. (74%) (Note 2). Ether is removed from the product by distillation. The residual material is cooled in liquid air, and 2.35 g. of bromine is vacuum-distilled into the flask. The mixture is warmed to –10° and shaken; the bromine reacts rapidly, leaving a colorless solution. The volatile unbrominated material is distilled *in vacuo* until the vapor pressure of the residue is less than 2 mm. (Note 3). The distillate is treated with 0.3 g. of bromine and 1 drop of water for 15 minutes at room temperature. Excess bromine, hydrogen bromide and alkyl bromides are removed by treatment of the mixture with 5 ml. of redistilled ethanolamine at room temperature. The product is then shaken with 1 ml. of 70% sulfuric acid at 0° (Note 4). Mass spectrometric analysis of the product shows the only impurity to be ordinary 2-methylpentane (26.5%).

(b) *2-Methylpentane-4-H₁²*. The Grignard reagent prepared from 18 g. of 2-bromo-4-methylpentane and 2.65 g. of magnesium in 40 ml. of ether is treated with water-H₂² as described above. The product, isolated and

freed of olefin as described for 2-methylpentane-2- H^2 , contains 19.6% of ordinary 2-methylpentane (Note 5).

(c) 2-Methylpentane-5- H_1^2 . The Grignard reagent prepared from an ether solution of 1-bromo-4-methylpentane is hydrolyzed with water- H_2^2 as described above. After the usual treatment with bromine and ethanolamine, the product is treated with 92% sulfuric acid at 0° for 5 minutes. The product is then vacuum-distilled from the sulfuric acid until the vapor pressure of the residue is 30 mm. at 0° . Finally the product is fractionated in a Podbielniak apparatus.

B. Notes

1. The preparation of 2-chloro-2-methylpentane, 2-bromo-4-methylpentane and 1-bromo-4-methylpentane is described by Stevenson, *et al.*

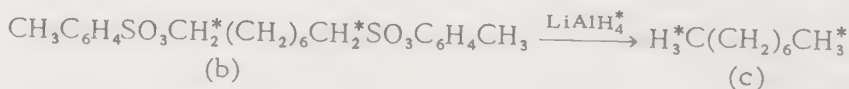
2. Mass spectrometric analysis indicated that this fraction contained 0.3% ethyl ether, 23% 2-methylpentenes and 18-25% ordinary 2-methylpentane.

3. By analysis, this product contained less than 0.46% olefin.

4. To remove any ammonia and residual ether.

5. A relatively nonvolatile liquid residue from the fractional distillation was probably 2,4,5,7-tetramethyloctane, which agrees with the elemental analysis and could be formed from two 1,3-dimethylbutyl radicals.

OCTANE-1,8- H_6^2



A. Streitwieser, Jr., J. Am. Chem. Soc., 77, 195 (1955).

A. Procedure

(a) 1,8-Octanediol-1,8- H_4^2 . To 2.0 g. of lithium aluminum hydride- H_4^2 in dry ether is added, with vigorous stirring, 10.1 g. of methyl suberate¹ dissolved in dry ether. Then, dilute hydrochloric acid is added until the inorganic salts separate, leaving a clear ether layer. The ether layer is separated, the salts are washed with ether, and the ether layers are combined. Evaporation of the ether leaves 7.8 g. of crude diol, and an additional 0.3 g. is obtained by continuous ether extraction of a dilute acid solution of the inorganic salts. Recrystallization of the product from benzene-hexane gives 5.9 g. (79%) of small white crystals, m.p. $56.0\text{--}58.0^\circ$. A second recrystallization from this solvent raises the m.p. to $58.0\text{--}58.5^\circ$.

(b) *Octamethylene-1,8-H₄² p-Toluenesulfonate*. A solution of 9.73 g. of 1,8-octanediol-1,8-H₄² in 100 ml. of pyridine is cooled to -10° , and 27.7 g. of *p*-toluenesulfonyl chloride is added. After the mixture is kept in an ice-salt bath for 1 hour with frequent mixing, 100 ml. of water is added in portions such that the temperature is kept below 10° . The white granular precipitate is collected and washed with water. The filtrate contains mono-*p*-toluenesulfonate ester (4 g.) as a heavy oil which, after drying and further treatment with 5.5 g. of *p*-toluenesulfonyl chloride in the same manner, yields additional product. After the combined products are dried, the yield of ester, m.p. $68.0-70.8^{\circ}$, is 24.9 g. (84%). The product may be recrystallized from benzene (Note 1).

(c) *Octane-1,8-H₆²*. The *p*-toluenesulfonate ester, 20.7 g., is placed in the thimble of a Soxhlet apparatus and extracted continuously with ether into a solution of 1.0 g. of lithium aluminum hydride-H₄² in dry ether. After 2 days, the hydride is apparently completely reacted. Dilute hydrochloric acid is added, and the ether layer is separated (Note 2). The ether solution is washed with water and dried with potassium carbonate. Ether is removed by distillation, and the residue is mixed with 100 ml. of pentane. Filtration of this mixture gives an additional 2.7 g. of unreacted ester. After removal of pentane from the filtrate, distillation of the residue from a small flask yields 0.6 ml. of a crude octane fraction, b.p. $115-124^{\circ}$ (Note 3). The combined octane-1,8-H₆² fractions from several runs are redistilled. The distillate is shaken with cold concentrated sulfuric acid (Note 4) and then washed with water and dried. Distillation of this product gives 1.1 ml. of octane-1,8-H₆², b.p. 121° , n_D^{25} 1.3945, d_4^{25} 0.7354.

B. Notes

1. After one recrystallization from benzene, octamethylene *p*-toluenesulfonate, prepared in the same manner, melted at $72.8-73.3^{\circ}$.
2. Filtration of the aqueous phase gave 3.4 g. of unreacted ester.
3. The considerable residue from the distillation was considered to be octyl-1,8-H₃² *p*-toluenesulfonate.
4. This treatment was not expected to cause exchange or isomerization of the octane (see Stevenson, *et al.*²).

C. Other Preparations

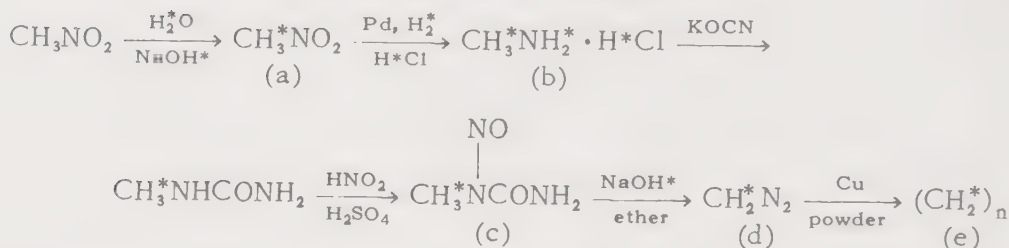
Bernhard, Gloor and Scheitlin³ have prepared octane-1,2-H₂² and octane-2,3-H₂² by hydrogenation of 1-octene and 2-octene, using a Raney nickel catalyst. By the same procedure, they have also prepared: decane-1,2-H₂², dodecane-1,2-H₂², tetradecane-1,2-H₂², hexadecane-1,2-H₂² and octadecane-1,2-H₂². However, see 2-methylpropane-2-H², Other Preparations.

¹A. I. Vogel, J. Chem. Soc., 1934, 333.

²D. P. Stevenson, C. D. Wagner, O. Beeck and J. W. Otvos, J. Am. Chem. Soc., 74, 3269 (1952).

³K. Bernhard, U. Gloor and E. Scheitlin, Helv. Chim. Acta, 35, 1908 (1952).

POLY(METHYLENE-H₂²)



L. C. Leitch, P. E. Gagnon and A. Cambron, Can. J. Research, 28B, 256 (1950).

A. Procedure

(a) *Nitromethane-H₃²*. Nitromethane-H₃² is prepared from sodium hydroxide-H² in water-H₂² and nitromethane by a method similar to that used by Wilson.¹

The nitromethane is first dried over phosphorus pentoxide and fractionated through an efficient column. A mixture of nitromethane (25 ml.) and 0.02 M sodium hydroxide-H² in water-H₂² (25 ml.) is heated for 24 hours at 110° in a sealed tube which is shaken mechanically. After cooling, the contents of the tube are transferred to a small separatory funnel. The lower layer of H²-nitromethane is separated, dried over phosphorus pentoxide and distilled under reduced pressure. The yield is 22.0 g; d₀²⁵ 1.1672 (Note 1). The H²-nitromethane is again treated with 0.02 M sodium hydroxide-H² in water-H₂² and purified as before. After a third treatment, the nitromethane-H₃² obtained is practically free of hydrogen, as shown by an infrared absorption spectrum. The over-all yield is 17.0 g. (59%) of nitromethane-H₃², d₀²⁵ 1.1832.

(b) *Methylamine-1-H₃² Hydrochloride*. A mixture of nitromethane-H₃² (6.0 g.) and water-H₂², which contains sufficient hydrogen-H² chloride to neutralize the methylamine formed, is treated with hydrogen-H₂², under 3 atmospheres pressure, in the presence of 0.4 g. of palladium-on-carbon catalyst.² When the reduction is complete, the solution is filtered and evaporated to dryness *in vacuo* (Note 2). The yield of crude methylamine-H₃² hydrochloride-H² is 5.9 g. (90%). The product, after recrystallization from hot butanol, melts at 227–228° (Note 3).

(c) *1-Methyl-H₃²-1-nitrosoarea*. Methylamine-1-H₃² hydrochloride, 23.0 g., is dissolved in 130 ml. of water, and 18.4 g. of potassium cyanate is

added. The solution is boiled gently for 15 minutes and then cooled to room temperature. To this solution of methyl- H_3^2 -urea is added 24.2 g. of 95% sodium nitrite. The solution is cooled to 0° and added slowly to a stirred solution of 23 g. of sulfuric acid in 100 ml. of water, maintained at 0° . The solid product is collected, washed with a little ice-water and dried in a desiccator. The yield of 1-methyl- H_3^2 -1-nitrosourea is 23.0 g. (60%).

(d) *Diazomethane- H_2^2* . A concentrated solution of sodium hydroxide- H^2 is prepared (Note 4) in a 100-ml. round-bottomed flask equipped with a stirrer and reflux condenser. About 100 ml. of absolute ether is added and then, with stirring, 5.3 g. (0.05 mole) of 1-methyl- H_3^2 -1-nitrosourea, in small portions. The ice-cold solution of diazomethane- H_2^2 in ether is separated in a funnel.

(e) *Poly(methylene- H_2^2)*. To a suspension of copper powder (Note 5) in a little ether is added slowly a solution of diazomethane- H_2^2 in ether. Evolution of nitrogen begins at once, and after one day the solution is colorless. The precipitate of copper powder and polymer is collected and digested on a steam-bath for a few hours with 40 ml. of 25% nitric acid. The white amorphous precipitate is collected and washed several times with hot water and then with 50% ethanol (Note 6). The yield of dry H^2 -polymethylene is 0.53 g. (53%); m.p. $122\text{--}123^\circ$ (Note 7).

B. Notes

1. The upper layer of water- H_2^2 is extracted with absolute ether, which is saved for extractions in later experiments. The water- H_2^2 is recovered by distillation; its density is now about 1.0600 instead of the initial 1.0970.

2. The water- H_2^2 recovered may be used with the same catalyst.

3. The butanol solvent contained appreciable amounts of butanol- H^2 after the recrystallization. Exchange occurred with the labile hydrogen- H^2 of the amino group and the hydrogen- H^2 chloride.

4. Clean sodium (5.0 g., 0.46 mole) is added in small portions to 30 ml. of water- H_2^2 which is cooled in a bath at 0° .

5. Zinc dust (6.0 g.) is added to a solution of copper sulfate pentahydrate (25.0 g.) in 250 ml. of water. After the mixture is stirred for one hour, the precipitated copper is allowed to settle, and the liquid is decanted. The copper powder is washed twice with water (200 ml.), then with methanol and finally with ether.

6. If the product retains a blue color it is resuspended in dilute nitric acid for a few minutes.

7. Analysis for carbon indicated a small amount of hydrogen in the compound; this was confirmed by the infrared spectrum.

C. Other Preparations

Wilson¹ prepared nitromethane- H_3^2 by exchange with water- H_2^2 using a modification of the procedure of Reitz.³ Repeated treatment was used rather than a large excess of water- H_2^2 .

Crawford and Fletcher⁴ prepared diazomethane- H_2^2 by the hydrolysis of 1-methyl- H_3^2 -1-nitroso-urea, also, but an attempted preparation *via* the decomposition of 4-methyl-4-(methyl- H_3^2 -nitrosoamino)-2-pentanone⁵ with sodium in benzyl alcohol resulted in loss of hydrogen- H^2 from the product through exchange with the solvent.

¹T. P. Wilson, J. Chem. Phys., 11, 361 (1943).

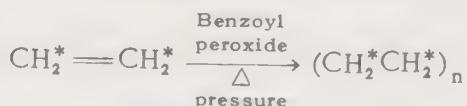
²Organic Syntheses, Vol. 26, Wiley, New York, 1946, p. 77.

³O. Reitz, Z. physik. Chem., A176, 363 (1935).

⁴B. L. Crawford and W. H. Fletcher, J. Chem. Phys., 19, 406 (1951).

⁵Organic Syntheses, Coll. Vol. III, Wiley, New York, 1955, p. 244.

POLY(ETHYLENE- H_4^2)



A. R. Ronzio, U. S. Atomic Energy Comm., Tech. Inform. Service, Oak Ridge, Tenn., LA-1478, 3-36 (1952); Chem. Abstracts, 48, 11290 (1954).

A. Procedure

The polymerization of ethylene- H_4^2 to form poly(ethylene- H_4^2) is carried out in a high pressure bomb constructed of stainless steel bar (Note 1). About 35 ml. of purified (Note 2) liquid ethylene- H_4^2 is distilled, *in vacuo*, into the bomb, which is cooled with liquid nitrogen. After the bomb is filled and is still immersed in liquid nitrogen, the inlet tube fitting attached to the lid is unscrewed, and a solution of 0.1 g. of benzoyl peroxide in 1 ml. of benzene is introduced into the reaction chamber with a hypodermic needle. The bomb is again closed, and the liquid nitrogen-bath is removed. The bomb is then placed in an electrically heated wax-bath, and the temperature of the bath is gradually raised to 240–265°. In a typical experiment, the initial pressure at 260–265° is about 9,200 p.s.i. and drops during 6 hours to 5000 p.s.i. (Note 3). The yield of poly(ethylene- H_4^2) is about 11 grams (60%) with a density in the range 1.02–1.05 and m.p. of 120–130°.

B. Notes

1. The bomb had the following dimensions: length, 25 cm.; diameter, 4.8 cm.; flange diameter, 7.3 cm.; flange thickness, 1.6 cm.; chamber

diameter, 1.6 cm.; chamber length, 22.7 cm. The bomb was fitted with a gauge reading to 15000 p.s.i., a closing valve and a safety disk. Diagrams and pictures of the apparatus are given in the report.

2. The ethylene- H_4^2 , which was prepared from acetylene- H_2^2 by chromous chloride reduction in hydrochloric acid- H^2 solution, was purified by passing it successively through a cold trap immersed in a Dry Ice-trichloroethylene mixture, a basic solution of mercuric oxide-potassium iodide complex, and a drying tube filled with calcium chloride.

3. As indicated by the final pressures in the bomb and the yields of polyethylene, polymerization was more complete with ethylene- H_4^2 than with ethylene.

ETHYLENE-1- H_2^2



A. Kruis and W. Schanzer, Z. physik. Chem., 191A, 301 (1942).

A. Procedure

The preparation of ethylene-1- H_2^2 by electrolysis is according to the method of Hölemann and Clusius¹ (Note 1). A 20% solution of propionic-2- H_2^2 acid in water is electrolyzed with bright platinum electrodes using a current density of 0.084 amperes per cm^2 . (see Figure XVI, 4) The heat of reaction is dissipated by cooling the solution with an ice-bath. The reaction is started with 2 g. of the acid in 11 g. of water, and more acid is added from a buret, as needed, to maintain the concentration. The addition of 1 g. of potassium hydroxide increases the conductivity, but the solution always remains acidic because of carbon dioxide formed in the reaction. The carbon dioxide is removed from the product by absorption in 25% potassium hydroxide solution (Note 2). The ethylene-1- H_2^2 is

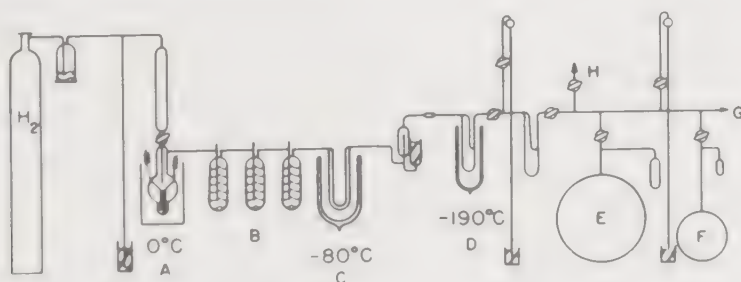


Fig. XVI, 4 Apparatus for the preparation of ethylene-1- H_2^2 (A. Kruis and W. Schanzer). A, electrolytic cell; B, scrubbers containing potassium hydroxide solution; C, cold trap for water removal; D, product receiver at -190° ; E and F, storage flasks; G, to glass fractionating column; H, to vacuum pump.

dried by passage through a trap at -80° and is collected in a trap cooled with liquid air. The product, which contains some ethane-1- H_2^2 and butane-2,3- H_4^2 , is rectified in a glass fractionating column. The separation of ethylene and ethane is effected in a bath of liquid ethylene (b.p. -104°) (Note 3). By the distillation procedure there are obtained: 2.4 l. of ethylene-1- H_2^2 , about 70 ml. of ethane-1- H_2^2 and about 200 ml. of butane-2,3- H_4^2 .

B. Notes

1. Hölemann and Clusius¹ have demonstrated that ethylene-1- H_2^2 is formed by the electrolysis of either propionic-3- H_3^2 acid or propionic-2- H_2^2 acid. Apparently the ethyl radical, formed by electrolysis, loses its odd hydrogen atom exclusively from the methyl group.

2. Removal of carbon dioxide from ethylene by fractionation is difficult; therefore, the product gas stream was passed through 3 scrubbers containing potassium hydroxide.

3. At this temperature ethane has a vapor pressure of about 300 mm. and does not form an azeotrope with ethylene. The purity of the distilled ethylene is followed by means of the vapor pressure at the temperature of melting hydrogen chloride (-112°). Only material is collected of which the vapor pressure agrees within ± 0.03 mm. so that a purity of about 98.7% appears assured. During the distillation of the pure ethylene, the pressure in the column remains constant, then drops and is again constant during the distillation of pure ethane. Finally, the butane is distilled from a liquid propane bath (b.p. -44°).

C. Other Preparations

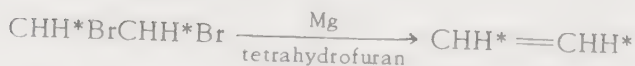
Ethylene-1- H_2^2 has been prepared² essentially according to the method described.

¹P. Hölemann and K. Clusius, Ber., 70B, 819 (1937).

²B. L. Crawford, Jr., J. E. Lancaster and R. G. Inskeep, J. Chem. Phys., 21, 679 (1953).

trans-ETHYLENE-1,2- H_2^2

METHOD I



W. M. Schubert, B. S. Rabinovitch, N. R. Larson and V. A. Sims, I. Am. Chem. Soc., 74, 4590 (1952).

A. Procedure (Note 1)

(a) *trans*-Ethylene-1,2- H_2^2 . Into an all-glass vacuum apparatus, consisting of a 50-ml. round-bottomed flask attached through a Dry Ice-type condenser to several traps, are introduced magnesium turnings (approximately 0.1 g.) and 15 ml. of tetrahydrofuran, freshly distilled from butylmagnesium bromide. The contents of the flask are frozen, placed under vacuum and melted several times to remove dissolved air. Under an atmosphere of purified nitrogen, a mixture of 95% *meso*- and 5% DL-1,2-dibromoethane-1,2- H_2^2 (about 0.03 ml.) is added to the frozen mixture. After the apparatus is again evacuated, the reaction mixture is allowed to melt in the dark, and the reaction proceeds at room temperature. The material collected in the liquid nitrogen-cooled traps is distilled from trap to trap a few times to ensure complete removal of solvent. Analysis (Note 2) indicates the product to be exclusively the *trans*-isomer.

Similar results are obtained in debrominations with zinc dust (approximately 0.4 g.) in 6 ml. of water run in the same way.

(b) *cis*- and *trans*-Ethylene-1,2- H_2^2 . Debrominations with sodium (0.06 g.) in liquid ammonia (20 ml.) are run in the above manner, except that the first trap contains dilute sulfuric acid for removal of entrained ammonia. With a mixture of 95% *meso*- and 5% DL-1,2-dibromoethane-1,2- H_2^2 , the product contains 57% *trans*-isomer; whereas, with a 50% *meso*- and 50% DL-1,2-dibromoethane-1,2- H_2^2 mixture, the product contains 50% of the *trans*-isomer (Note 3).

B. Notes

1. Schubert, *et al.*, discuss the debromination of vicinal dibromides, with the divalent metals, in terms of an ionic mechanism; those with sodium in terms of a free radical mechanism.

2. The percentages of *cis*- and *trans*-olefin in the ethylene-1,2- H_2^2 reaction product were obtained from the infrared absorption¹ at 987 $cm.^{-1}$ (*trans*) and 842 $cm.^{-1}$ (*cis*). Known mixtures of *trans*- and *cis*-isomers were used to obtain an empirical calibration of mixture composition *vs.* transmission at the two frequencies.

3. Explanations for this difference are discussed by the authors.

C. Other Preparations

cis-Ethylene-1,2- H_2^2 has been prepared,² in 60% yield, by the debromination of *meso*-1,2-dibromoethane-1,2- H_2^2 with iodide ion in methanol. As predicted by the reaction mechanism proposed by Hine and Brader,³ the net steric course of the reaction in 90% methanol at 59° was one of ex-

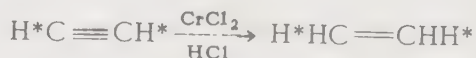
clusive *cis* elimination. *trans*-Ethylene-1,2- H_2^2 was practically unaffected under these conditions.

¹R. L. Arnett and B. L. Crawford, J. Chem. Phys., 18, 118 (1950).

²W. M. Schubert, H. Steadly and B. S. Rabinovitch, J. Am. Chem. Soc., 77, 5755 (1955).

³J. Hine and W. H. Brader, Jr., *ibid.*, 77, 361 (1955).

METHOD II



W. I. Patterson and V. du Vigneaud, J. Biol. Chem., 123, 327 (1938).

A. Procedure

Acetylene- H_2^2 is reduced to ethylene-1,2- H_2^2 with a solution of chromous chloride¹ prepared by the reduction of 2 pounds of chromic chloride ($CrCl_3 \cdot 6H_2O$) in 1800 ml. of 25% hydrochloric acid solution with 1 pound of mossy zinc. The chromous chloride solution is added to a 20-liter bottle containing acetylene- H_2^2 at less than one atmosphere pressure. The bottle and contents are then shaken vigorously for 6 hours. The solution is used to treat a second bottle of the gas in the same manner.

B. Other Preparations

Ethylene-1,2- H_2^2 has been prepared by the action of zinc² on 1,2-dibromoethane-1,2- H_2^2 (see ethylene- H_4^2), by reduction of acetylene- H_2^2 with chromous chloride,³⁻⁵ and by the reduction of acetylene- H_2^2 with a number of chemical reagents.⁶ Of these, the following gave entirely or largely the *cis*-isomer: zinc-1*N* alcoholic hydrogen chloride, magnesium-1*N* alcoholic hydrogen chloride, copper activated zinc-water, palladium activated zinc-alcohol, copper activated zinc-1*N* hydrochloric acid, and copper activated magnesium-1*N* hydrochloric acid. The following reagents gave *trans*-ethylene-1,2- H_2^2 : chromous chloride-acid, sodium-ammonia, and magnesium-ammonium chloride.

Attempts to prepare *cis*-ethylene-1,2- H_2^2 by the reduction of acetylene with a palladium catalyst⁷ and with either nickel or palladium catalysts⁵ resulted in mixtures of all the possible deuterated ethylenes. It was shown⁵ that the reaction of hydrogen- H_2^2 and acetylene on a nickel-kieselguhr catalyst at -80° yields a preponderance of the *cis*-isomer; however, acetylene exchanged rapidly with itself and with residual hydrogen on the catalyst, thus giving a mixture of deuterated ethylenes.

¹W. Traube and W. Passarge, Ber., 49, 1692 (1916).

²M. de Hemptinne, J. Jungers and J. M. Delfosse, J. Chem. Phys., 6, 319 (1938).

³G. B. Kistiakowsky and W. L. Marshall, J. Am. Chem. Soc., 74, 88 (1952).

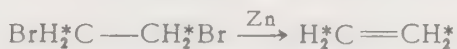
⁴H. J. Bernstein, A. D. E. Pullin, B. S. Rabinovitch and N. R. Larson, J. Chem. Phys., 20, 1227 (1952).

⁵J. E. Douglas and B. S. Rabinovitch, J. Am. Chem. Soc., 74, 2486 (1952).

⁶B. S. Rabinovitch and F. S. Looney, *ibid.*, 75, 2652 (1953).

⁷R. L. Arnett and B. L. Crawford, J. Chem. Phys., 18, 118 (1950).

ETHYLENE-H₂



L. C. Leitch and A. T. Morse, Can. J. Chem., 30, 924 (1952).

A. Procedure

The apparatus consists of a two-necked flask equipped with a dropping funnel and a vertical condenser; the latter is connected directly to a manifold which includes a cold trap and is connected to a storage flask (Figure XVI, 5). Zinc dust, 40.0 g., and a crystal of sodium iodide are placed in the reaction flask, and the apparatus is evacuated. The cold trap is immersed in a Dry Ice-acetone bath, and a solution of 38.4 g. of 1,2-dibromoethane-H₂ in 50 ml. of dioxane is placed in the dropping fun-

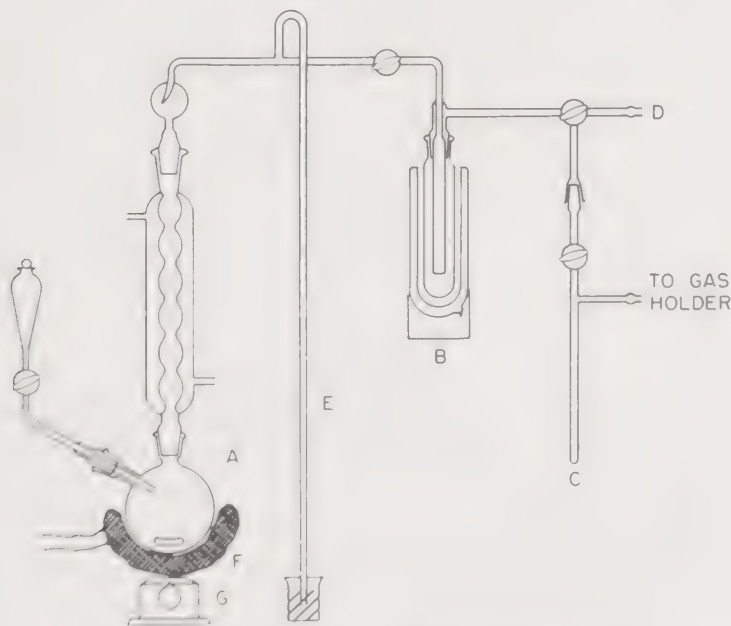


Fig. XVI, 5 Apparatus for preparation of ethylene-H₂ (L. C. Leitch and A. T. Morse). A, reaction flask; B, cold trap at -78° ; C, cold trap for collection of last of product at liquid nitrogen temperature; D, to vacuum; E, manometer; F, heating mantle; G, magnetic stirring motor.

nel. A few ml. of the solution is added slowly to the zinc, and the mixture is heated gently with stirring. When the ethylene- H_4^2 evolved is at a pressure of nearly 1 atmosphere, it is collected in a storage flask over mercury. The contents of the funnel are then added dropwise while the suspension is slowly heated to boiling. At the end of the experiment, the ethylene- H_4^2 in the apparatus is frozen in the trap with liquid nitrogen and then distilled into the storage flask. The yield of ethylene- H_4^2 is 4.0-4.3 l. (80-86%).

B. Other Preparations

Ethylene- H_4^2 has been prepared by the reaction of zinc with 1,2-dibromoethane- $\text{H}_4^{2,1,2}$ in dioxane³ or methanol.⁴

Ethylene- H_4^2 has also been prepared⁵ by the addition of hydrogen- H_2^2 to acetylene- H_2^2 on a nickel catalyst and by the reduction⁶ of acetylene- H_2^2 with a chromous chloride solution in water- H_2^2 containing hydrogen- H^2 chloride.

¹M. de Hemptinne, J. Jungers and J. M. Delfosse, *J. Chem. Phys.*, 6, 319, (1938); *Nature*, 140, 323 (1937).

²G. Joris, H. S. Taylor and J. C. Jungers, *J. Am. Chem. Soc.*, 60, 1982 (1938).

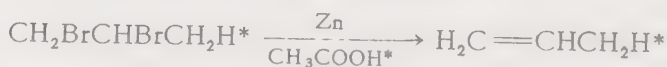
³C. L. Wilson and A. W. Wylie, *J. Chem. Soc.*, 1941, 596.

⁴W. S. Gallaway and E. F. Barker, *J. Chem. Phys.*, 10, 88 (1942).

⁵J. E. Douglas and B. S. Rabinovitch, *J. Am. Chem. Soc.*, 74, 2486 (1952).

⁶A. R. Ronzio, U. S. Atomic Energy Comm., Tech. Inform. Service, Oak Ridge, Tenn., LA-1478, 3-36 (1952); *Chem. Abstracts*, 48, 11290 (1954).

PROPENE-3- H_1^2



C. D. Hurd and J. L. Azorlosa, *J. Am. Chem. Soc.*, 73, 33 (1951).

A. Procedure

In a flask equipped with a dropping funnel, a mercury-sealed stirrer and a condenser, a suspension of 14 g. of zinc dust in 200 ml. of alcohol is heated to boiling. With vigorous stirring, 14 g. of 1,2-dibromopropane-3- H_1^2 is dropped slowly into the mixture. The evolved propene-3- H_1^2 is conducted through a stopcock into a tube at -78° , which is attached to a Davis column, the reflux head of which is also at -78° . When the addition is complete, heating of the mixture is continued for 0.5 hour. Then the stopcock is closed, and the cooling bath is removed from the tube containing the propene-3- H_1^2 . When reflux is established in the column, the product is distilled, b.p. -44 to -43° , collected, and stored over brine in a 5-l. bottle. Atmospheric pressure is maintained through-

out the distillation by adjustment of a leveling bottle connected to the gas-holder. The volume of product is 1200 ml.; yield, 78%.

B. Other Preparations

Propene-1-H₁² has been prepared¹ by the reduction of propyne-1-H₁² with copper activated zinc and 1 N hydrochloric acid.

Propene-H₆² has been prepared² by the partial reduction of propyne-H₄² with hydrogen-H₂² over a palladium-charcoal catalyst according to the procedure of Baker.³

¹B. S. Rabinovitch and F. S. Looney, J. Am. Chem. Soc., 75, 2652 (1953).

²R. C. Lord and P. Venkateswarlu, J. Opt. Soc. Am., 43, 1079 (1953).

³A. W. Baker, thesis, Mass. Institute of Technology, September, 1950.

PROPADIENE-H₄² (Allene-H₄²)



R. C. Lord and P. Venkateswarlu, J. Chem. Phys., 20, 1240 (1952).

A. Procedure

A mixture of propyne-H₄² and allene-H₄² is obtained by the reaction of water-H₂² vapor and magnesium carbide at 350° (Note 1); see propyne-H₄². The allene-H₄² is separated from propyne-H₄² by slowly passing a mixture of the two gases through an ammoniacal silver nitrate solution¹ (Note 2). The allene-H₄² is then passed through dilute hydrochloric acid to remove ammonia and finally through water. The product is passed through a drying train (Note 3); from about 40 ml. of liquid mixed isomers, 2 ml. of liquid allene-H₄² is recovered.

B. Notes

1. Allene-H₄² was obtained as a by-product in the preparation of propyne-H₄², with which it is isomeric. It was noted² that there was an increase in the relative amount of allene-H₄² when the reaction was carried out at higher temperatures (up to 350°). Investigation of the thermodynamics of the isomerization of methylacetylene to allene showed that increased temperature does indeed favor this conversion. Experimental evidence¹ indicated that the two isomers are not formed from magnesium carbide in equilibrium amounts. Although the calculated equilibrium at 150° calls for about 10% allene, apparently less than 1% is actually formed. As the temperature is increased, the percentage of allene goes up, the ob-

served rate of increase being much larger than the calculated. However, it may simply be that the higher temperature facilitates isomerization. At the highest temperature where measurement was made, the observed allene concentration was still smaller than calculated by a factor of three.

2. Overend and Thompson³ also prepared allene- H_4^2 , by the reaction described, at 350° in a vacuum system. They removed propyne- H_4^2 by passing the gaseous mixture through an aqueous solution of potassium iodide containing mercuric chloride and then through ammoniacal silver nitrate solution.

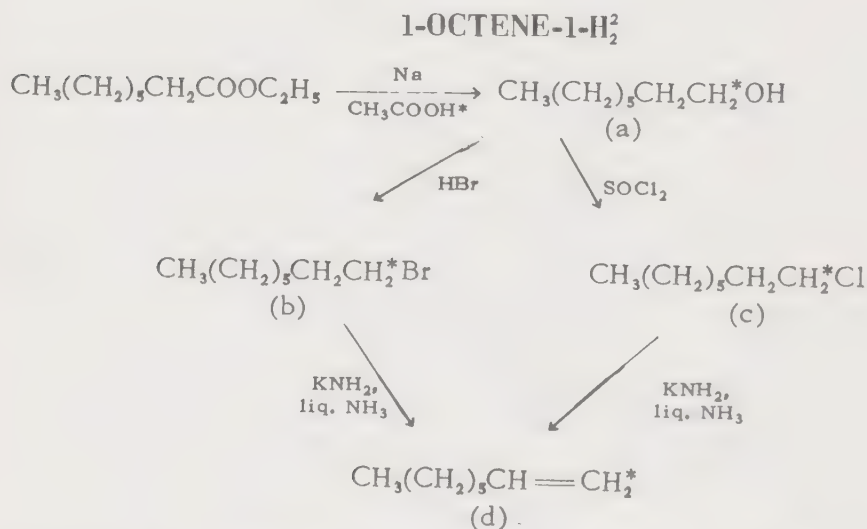
3. Leitch and Renaud⁴ used phosphorus pentoxide.

¹J. Ocampo, thesis, Massachusetts Institute of Technology, September, 1950.

²A. W. Baker, thesis, "The Preparation of Cyclopropane- H_6^2 , etc.," Massachusetts Institute of Technology, September, 1950.

³J. Overend and H. W. Thompson, J. Opt. Soc. Am., 43, 1065 (1953).

⁴L. C. Leitch and R. Renaud, Can. J. Chem., 30, 79 (1952).



D. G. Hill, W. A. Judge, P. S. Skell, S. W. Kantor and C. R. Hauser, J. Am. Chem. Soc., 74, 5599 (1952).

A. Procedure

(a) *1-Octanol-1- H_2^2* . Ethyl octanoate is dissolved in 6-10 times its volume of anhydrous ether which contains a trace of phenolphthalein as an indicator. The solution is cooled to about -5° , and 4 equivalents each of sodium (in small pieces) and acetic acid- H^2 (Note 1) are added to the stirred solution simultaneously and very slowly (Note 2). After the reaction is complete, cold water is added cautiously. The ether layer is separated, and the ether is removed on a steam-bath. The residue is heated with ethanolic potassium hydroxide (Note 3). After this

mixture cools, the product is extracted with ether and dried. The solution is filtered, ether is removed, and the product is distilled.

(b) *1-Bromoöctane-1-H₂²*. 1-Octanol-1-H₂² is converted to 1-bromoöctane-1-H₂² with gaseous hydrogen bromide at 100°. ¹

(c) *1-Chloroöctane-1-H₂²*. 1-Octanol-1-H₂² is converted to 1-chloroöctane-1-H₂² with thionyl chloride according to the method of Clark and Streight. ²

(d) *1-Octene-1-H₂²*. The halide (0.1-0.2 mole), usually in anhydrous ether, is added to a stirred solution of one equivalent (or a slight excess) of potassium amide in 100-150 ml. of liquid ammonia. When the reaction is finished, an excess of solid ammonium chloride is added. Water is then added, the ammonia is evaporated, and the mixture is extracted with ether. The ether solution is washed successively with water, dilute hydrochloric acid and water. It is then dried over Drierite and fractionated to isolate the olefin (Note 4).

(e) *1-Heptanol-1-H₂²*. Ethyl heptanoate is reduced with sodium and acetic acid-H² according to the above procedure for 1-octanol-1-H₂².

(f) *1-Bromoheptane-1-H₂²*. 1-Heptanol-1-H₂² is treated with gaseous hydrogen bromide at 100°. ¹

(g) *Octanoic-2-H₂² Acid*. Heptylmagnesium-1-H₂² bromide is prepared in ether solution and carbonated with carbon dioxide (see carbon-14 labeled acids).

(h) *1-Octanol-2-H₂²*. The reduction of octanoic-2-H₂² acid with lithium aluminum hydride is performed according to the method of Nystrom and Brown³ (see Brown). ⁴

(i) *1-Bromoöctane-2-H₂²*. 1-Octanol-2-H₂² is treated with gaseous hydrogen bromide at 100°. ¹

(j) *1-Chloroöctane-2-H₂²*. 1-Octanol-2-H₂² is treated with thionyl chloride as in the preparation of 1-chloroöctane-1-H₂².

(k) *1-Octene-2-H²*. 1-Bromo- or 1-chloroöctane-2-H₂² is treated with potassium amide as described for 1-octene-1-H₂² (Note 4).

(l) *2-Ethyl-1-butanol-1-H₂²*. Ethyl 2-ethylbutyrate is reduced with sodium and acetic acid-H² as in the preparation of 1-octanol-1-H₂².

(m) *1-Bromo-2-ethylbutane-1-H₂²*. 2-Ethyl-1-butanol-1-H₂² is treated with gaseous hydrogen bromide at 100°. ¹

(n) *2-Ethyl-1-butene-1-H₂²*. 1-Bromo-2-ethylbutane-1-H₂² is treated with potassium amide as in the preparation of 1-octene-1-H₂².

(o) *Ethyl 2-Ethylbutyrate-2-H²*. Ethyl 2-ethylbutyrate is stirred with an ether suspension of 1 equivalent of triphenylmethylpotassium⁵ for 2 hours. Then a slight excess of water-H₂² is added, and the ester is isolated.

(p) *2-Ethyl-1-butanol-2-H²*. Ethyl 2-ethylbutyrate-2-H² is reduced with sodium and acetic acid-H²; see 1-octanol-1-H₂² above (Note 5).

(q) *1-Bromo-2-ethylbutane-2-H²*. 2-Ethyl-1-butanol-2-H² is treated with gaseous hydrogen bromide at 100°. ¹

B. Notes

1. This reduction is done according to the method of Prins,⁶ modified to avoid all traces of water.

2. The solution should always be slightly acidic.

3. This is to saponify any unchanged ester.

4. This study of the elimination reaction of alkyl halides with potassium amide has shown that 1-bromo-2-ethylbutane exhibits β -elimination, i.e., removal of hydrogen halide involving a β -hydrogen atom; whereas, octyl halides exhibit α - as well as β -elimination. Hydrogen halide was eliminated more rapidly than hydrogen- H^2 halide, as is generally the case.^{7,8} In competition with the elimination reactions, amines were formed by displacement reactions. 2-Ethylbutylamine-1- H^2 and 2-ethylbutylamine-2- H^2 were formed to the extent of about 20%. Octylamine-1- H^2 and octylamine-2- H^2 were formed to the extent of about 80%.

5. Oxidation of a sample of the 2-ethyl-1-butanol-2- H^2 with basic permanganate gave 2-ethylbutyric-2- H^2 acid of practically the same deuterium content as the alcohol.

¹*Organic Syntheses*, Vol. 15, Wiley, New York, 1935, p. 24.

²R. H. Clark and H. R. L. Streight, *Trans. Roy. Soc. Canada*, [3] 23, Sec. 3, 77 (1929).

³R. F. Nystrom and W. G. Brown, *J. Am. Chem. Soc.*, 69, 2548 (1947).

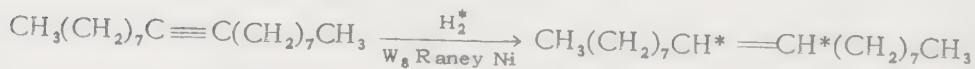
⁴*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 469.

⁵R. Levine, E. Baumgarten and C. R. Hauser, *J. Am. Chem. Soc.*, 66, 1230 (1944).

⁶H. F. Prins, *Rec. trav. chim.*, 42, 1050 (1923).

⁷C. L. Wilson, *J. Chem. Soc.*, 1936, 1550.

⁸W. F. K. Wynne-Jones, *J. Chem. Phys.*, 2, 381 (1934).

9-OCTADECENE-9,10- H^2 

N. A. Khan, *J. Am. Chem. Soc.*, 74, 3018 (1952).

A. Procedure

A solution of 5 g. of 9-octadecyne in 125 ml. of purified dioxane is hydrogenated in the presence of deuterized Raney nickel (Note 1). The reaction is very selective, and hydrogen- H^2 absorption ceases when 1 mole per mole of acetylenic compound is consumed (Note 2). After removal of the catalyst by filtration, the solution is evaporated, and the crude product is dissolved in pure, dry ether to obtain a 10% solution. This solution is cooled to -40 to -50° for 2 hours, and, after removal of a first crop of crystals, the filtrate is again cooled to -45 to -50° for

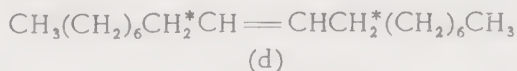
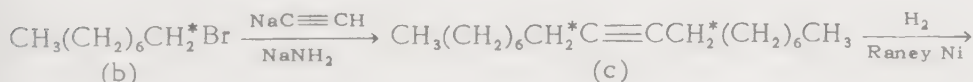
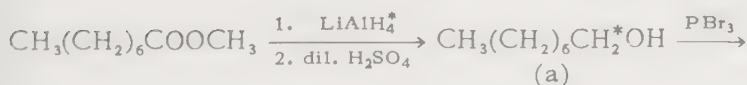
2 hours more. After removal of a second crop of crystals, the final filtrate is distilled. The 9-octadecene-9,10- H_2^2 is distilled under vacuum, b.p. 140–142° (2–3 mm.).

B. Notes

1. For the preparation of this special catalyst, designated as W_8 , see oleic-9,10- H_2^2 acid.

2. The fact that deuteration of this symmetrical acetylenic hydrocarbon stops after reduction of the triple bond to a double bond indicates that nickel forms a stronger association compound with the resultant double bond because of the equal distribution of electrons contributed by the balanced alkyl groups on either side. This association compound is probably not vulnerable to attack by deuterium on the surface of the catalyst.

9-OCTADECENE-8,11- H_4^2



R. A. Max and F. E. Deatherage, J. Am. Oil Chemists Soc., 28, 110 (1951).

A. Procedure

(a) *1-Octanol-1- H_2^2* . Methyl caprylate is reduced with lithium¹ aluminum hydride- H_4^2 according to the method of Nystrom and Brown.² Following hydrolysis of the lithium aluminum alkoxide with dilute sulfuric acid, the ether layer is separated, dried and distilled; yield 80–90%.

(b) *1-Bromoöctane-1- H_2^2* . To 20 g. (0.154 mole) of 1-octanol-1- H_2^2 in a 3-necked 500-ml. flask equipped with dropping funnel, stirrer, and condenser attached to a hydrobromic acid trap, is added gradually with stirring 38.0 g. (0.1 mole) of phosphorus tribromide. The reaction mixture is heated under reflux, with an oil-bath at 120°, for 8 hours. After cooling, the flask contents are transferred to a separatory funnel and washed with three portions of concentrated sulfuric acid. The combined washings are extracted with 100 ml. of petroleum ether, and the resulting extract is added to the crude bromide. The crude bromide mixture is washed with water, sodium carbonate solution, and then with water to neutrality.

After drying the solution over anhydrous sodium sulfate, the ether is removed, and the bromide is distilled, b.p. 99° (32 mm.); yield, 85-95%.

(c) *9-Octadecyne-8,11- H_4^2* . 1-Bromoöctane-1- H_2^2 , 40 g. (0.2 mole), is placed in a Parr bomb, which is then closed and cooled in Dry Ice. Sodium amide,³ 3.9 g. (0.1 mole), and sodium acetylide,³ 4.8 g. (0.1 mole), are weighed into small bottles, which are stoppered and cooled in Dry Ice. When the temperature of the bomb and contents is below -40° , the bomb is opened, and 75 ml. of liquid ammonia, the sodium amide and the sodium acetylide are added quickly. The bomb is immediately closed. The temperature of the bomb and contents gradually increases and exceeds room temperature. Within an hour the temperature begins to drop, and the bomb is gently agitated for 36-48 hours. The bomb is then cooled in Dry Ice and opened, and 50 ml. of water is added dropwise during 30-50 minutes. This mixture is transferred to a 1-l. separatory funnel with 300 ml. of petroleum ether; the aqueous phase is separated and extracted with petroleum ether. The combined extract is washed 4-6 times with 1 *N* hydrochloric acid and then with water until neutral. Solvent is removed from the dried extract, and the residue is fractionally distilled. After the forerun of 1-decyne-3- H_2^2 boiling at $50-65^{\circ}$ (32 mm.), 13-17 g. of *9-octadecyne-8,11- H_4^2* is collected at $142-145^{\circ}$ (2 mm.); d^{29} 0.795, n^{29} 1.4475, iodine number 98 (Note 1).

(d) *cis-9-Octadecene-8,11- H_4^2* . To 5 g. of *9-octadecyne-8,11- H_4^2* , dissolved in 35 ml. of cyclohexane and placed in a semi-micro hydrogenator,⁴ is added 0.5 g. of W₁ Raney nickel catalyst. One molar equivalent of hydrogen is absorbed (Note 2). The solvent is removed, 50 ml. of ether is added, and this solution is cooled to -40 to -50° . About 0.5 g. of crystalline by-product is removed. After removal of solvent, the product distills at $138-148^{\circ}$ (2 mm.). The yield of *cis-9-octadecene-8,11- H_4^2* is 3-4 g.; d^{31} 0.795, n^{31} 1.4431.

B. Notes

1. *9-Octadecyne* can be prepared by carrying out the reaction in one or two stages at atmospheric pressure and using a Dry Ice-condenser for ammonia reflux.⁵ This procedure is long, and the yields are lower than with the bomb. The best yields are obtained when the temperature in the bomb increases to about 70° and is maintained there for a few hours.

2. The hydrogenation was very selective, and hydrogen absorption stopped when 1 mole of hydrogen per mole of acetylenic compound was taken up. When platinum oxide catalyst⁶ was used there was a sharp drop in rate of hydrogen uptake after 1 mole of hydrogen per mole of compound was absorbed.

⁴Metal Hydrides, Inc., Beverly, Mass.

²R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 1197 (1947).

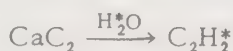
³*Inorganic Syntheses*, Vol. II, McGraw-Hill, New York, 1946, pp. 75-81, 128-135.

⁴L. H. Joshel, *Ind. Eng. Chem.*, 15, 590 (1943).

⁵A. L. Henne and K. W. Greenlee, *J. Am. Chem. Soc.*, 67, 484 (1945).

⁶*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

ACETYLENE-H₂²



F. W. Breuer, *J. Am. Chem. Soc.*, 58, 1289 (1936).

A. Procedure (Note 1)

Especially pure calcium carbide (Note 2) is crushed with exclusion of moisture, and the inner cores are powdered in an atmosphere of dry nitrogen. The generator is charged with 8 g. (0.13 mole) of carbide which is covered with a 3-mm. layer of ignited quartz sand. In the side-arm of the generator, which is connected to a manifold, a section of powdered calcium carbide is secured between plugs of glass wool. The thoroughly dried acetylene generating and storage part of the system is outgassed while the carbide is heated to 250°. In the dropping funnel attached to the generator is placed 4 ml. (0.22 mole) of water-H₂², *d*₂²⁰ 1.1071, which is added slowly to the carbide. The acetylene-H₂² is collected over mercury in the storage bulbs, such that the pressure is kept close to atmospheric, as indicated by a manometer. Water-H₂² is added in small portions when no change in pressure is noted during 0.5 hour (Note 3). Unreacted water-H₂² is driven into fresh carbide in the side-arm by heating the generator with an oil-bath at 170°. By cooling the storage bulbs with Dry Ice while heating the generator, a nearly quantitative yield of acetylene-H₂² is obtained (Note 4).

B. Notes

1. A diagram of the apparatus used is given by Breuer.
2. Especially pure calcium carbide was obtained from Carbide and Carbon Chemicals Corporation.
3. The total addition took 60 hours.
4. In this procedure, one-half of the hydrogen-H² from the water-H₂² remains as calcium hydroxide-H₂². This may be made to react with fresh carbide by dehydrating the calcium hydroxide-H₂² above 580°.

C. Other Preparations

Ronzio¹ and Patterson and du Vigneaud² have prepared acetylene-H₂² on a large scale. In the latter case, 100 g. of calcium carbide and 25 g.

of water- H_2^2 were used. The calcium hydroxide- H_2^2 formed was repeatedly dehydrated, and the water- H_2^2 again reacted with fresh calcium carbide until only a small amount of hydrogen- H^2 remained as calcium hydroxide- H_2^2 . Acetylene- H_2^2 has been prepared on numerous occasions³⁻¹⁵ from calcium carbide and water- H_2^2 .

¹A. R. Ronzio, U. S. Atomic Energy Comm., Tech. Inform. Service, Oak Ridge, Tenn., LA-1478, 3-36 (1952); Chem. Abstracts, 48, 11290 (1954).

²W. I. Patterson and V. du Vigneaud, J. Biol. Chem., 123, 327 (1938).

³H. Erlenmeyer, O. Bitterlin and H. M. Weber, Helv. Chim. Acta, 22, 701 (1939).

⁴F. Stitt, J. Chem. Phys., 7, 297 (1939).

⁵B. D. Saksena, J. Chem. Phys., 20, 95 (1952).

⁶J. E. Douglas and B. S. Rabinovitch, J. Am. Chem. Soc., 74, 2486 (1952).

⁷G. A. Hornbeck and R. C. Herman, Ind. Eng. Chem., 43, 2739 (1951).

⁸R. Friedman and E. Burke, Ind. Eng. Chem., 43, 2772 (1951).

⁹L. C. Leitch and A. T. Morse, Can. J. Chem., 30, 924 (1952).

¹⁰J. C. Jungers and J. Verhulst, Acad. roy. Belg., Classe sci., Mem., 23, 3 (1949).

¹¹F. L. Mohler, V. H. Dibeler, L. Williamson and H. Dean, J. Research Natl. Bur. Standards, 48, 188 (1952).

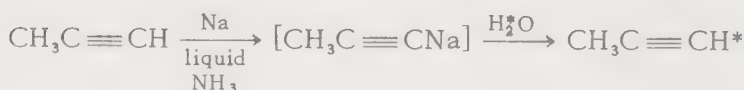
¹²E. D. Hostrand and A. B. F. Duncan, J. Am. Chem. Soc., 76, 3377 (1954).

¹³F. Stitt, J. Chem. Phys., 8, 56 (1940).

¹⁴J. Goubeau, H. Luther, K. Feldmann and G. Brandes, Ber., 86, 214 (1953).

¹⁵E. C. Wingfield and J. W. Straley, J. Chem. Phys., 23, 731 (1955).

PROPYLENE-1- H^2



B. S. Rabinovitch and F. S. Looney, J. Am. Chem. Soc., 75, 2652 (1953).

A. Procedure

Propyne (Note 1) is dissolved with a stoichiometric amount of sodium in liquid ammonia; the ammonia is evaporated, and the sodium methylacetylide is dried under vacuum for 12 hours. Then, a small excess of water- H_2^2 is added dropwise to the sodium compound, and the evolved propyne-1- H^2 is collected in a trap, which is cooled with liquid nitrogen, and purified by distillation (Note 2).

B. Notes

1. The preparation of propyne from sodium acetylide and methyl iodide in liquid ammonia is described by Rabinovitch and Looney.

2. Infrared analysis of the product indicated approximately 10% of propyne impurity.

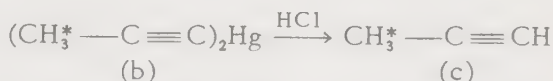
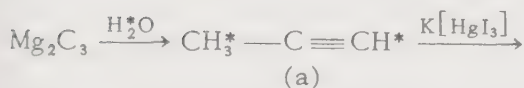
C. Other Preparations

Propyne-1- H^2 has been prepared¹ by the action of hydrogen- H^2 chloride on di-1-propynylmercury prepared according to the procedure of Leitch and Renaud² (see propyne-3- H_3^2). The product contained very little ordinary propyne.

¹R. J. Grisenthwaite and H. W. Thompson, Trans. Faraday Soc., 50, 212 (1954).

²L. C. Leitch and R. Renaud, Can. J. Chem., 30, 79 (1952).

PROPYNE-3- H_3^2



L. C. Leitch and R. Renaud, Can. J. Chem., 30, 79 (1952).

A. Procedure

(a) *Propyne- H_4^2* . Crude magnesium carbide, 40.0 g. (Note 1), is placed in a 500-ml. 3-necked flask fitted with a dropping funnel, an inlet tube for introducing argon, a stirrer with a mercury seal, and a short, jacketed Vigreux column. A spiral trap cooled to -78° is attached to the side-arm of the column. The carbide is moistened with dioxane (Note 2), and a solution of 8 ml. of water- H_2^2 (99.7 mole %) in 30 ml. of dry dioxane is added dropwise with stirring over a period of 2 hours. The reaction mixture warms and ultimately refluxes from the heat of the reaction. Additional amounts of the solution are added until no further evolution of heat occurs (Note 3). The reaction mixture is finally refluxed for 1 hour while a slow stream of argon is passed through the apparatus. The propyne- H_4^2 is then distilled over phosphorus pentoxide on a vacuum line and is freed of traces of acetylene by fractional distillation from a bath at -40° . The yield, measured at -78° , is 16.0 ml. (about 95%, Note 4).

(b) *Bis(1-propynyl- H_3^2)mercury*. In a flask attached to a vacuum line, 10-15 ml. of liquid propyne- H_4^2 is frozen with liquid nitrogen. The apparatus is evacuated, and the propyne is allowed to melt and evaporate slowly into a flask containing 150 ml. of alkaline potassium mercuric iodide solution, which is continuously agitated. The precipitate of insoluble white mercury derivative (Note 5) is collected and washed several times with water and finally with acetone. The product is purified by continuous extraction in a Soxhlet apparatus with acetone, from which it separates in white, lustrous needles, m.p. $202-204^\circ$. The yield is nearly quantitative (Note 6).

(c) *Propyne-3-H₃²*. To 20.0 g. of bis(1-propynyl-H₃²)mercury in an apparatus similar to that used to prepare propyne-H₄², 120 ml. of 6 *N* hydrochloric acid is added dropwise. The propyne-3-H₃² is condensed in a receiver cooled to -78°. When all the solid has been decomposed, the propyne-3-H₃² which remains in the apparatus is swept out with a stream of argon. The product is purified by distillation over phosphorus pentoxide on a vacuum line. The yield is 6.2 ml. (78%), measured at -78° (Note 7).

B. Notes

1. The preparation of magnesium carbide is given by Leitch and Renaud.

2. The dioxane was dried over sodium and distilled.

3. A total of about 100 ml. of solution is required.

4. Unreacted water-H₂² and dioxane are recovered for re-use. The yield of propyne-H₄² was based on water-H₂² actually consumed.

5. Because of the obnoxious and persistent odor of di-1-propynylmercury, use of a hood is recommended, and contact with the mercurial is avoided by wearing rubber gloves.

6. Pure propyne-H₄² is regenerated from the mercury compound by hydrolysis with a solution of hydrogen-H² bromide in water-H₂². The yield of pure propyne-H₄² is 81.9%, after redistillation through phosphorus pentoxide *in vacuo*.

7. The vapor pressures of propyne, propyne-3-H₃² and propyne-H₄² are compared by Leitch and Renaud over a temperature range from -24° to 63.5°.

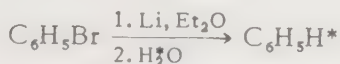
C. Other Preparations

The hydrolysis of magnesium carbide with water-H₂² containing various amounts of excess deuterium has been used to prepare¹ propyne-3-H₁², propyne-3-H₂², propyne-1,3-H₂², propyne-1,3-H₃² and propyne-H₄². Propyne-3-H₃² was prepared from the latter compound by exchange with a large excess of aqueous sodium hydroxide. Propyne-1-H² was prepared in high concentration by exchange at 40° of pure propyne with excess water-H₂² containing sodium hydroxide-H².

¹L. F. Thomas, E. I. Sherrard and J. Sheridan, *Trans. Faraday Soc.*, 51, 619 (1955).

BENZENE-H₁²

METHOD I

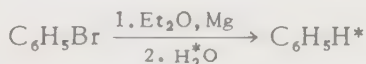


W. M. Lauer and W. E. Noland, *J. Am. Chem. Soc.*, 75, 3689 (1953).

A. Procedure

Lithium sand, 16.8 g., (Note 1) is suspended in 300 ml. of anhydrous ether. Over a period of 3 hours, 157.3 g. (1.0 mole) of bromobenzene in 225 ml. of dry ether is added dropwise with vigorous stirring. More lithium is added during the addition of halide in order to maintain a slight excess. After all the bromobenzene is introduced, the mixture is refluxed, with stirring, for 1.5 hours. Then, over a period of 1.5 hours, 25.8 g. (1.43 moles) of water-H₂² is added, with stirring, to the solution of phenyllithium. After the water is added, the mixture is refluxed with stirring for 1 hour. The precipitate is collected, and the filtrate is distilled through a Vigreux column (Note 2). The product is fractionated (Note 3), and after removal of ether and a forerun of 5.13 g., b.p. 78–78.2°, 32.9 g. (41.6%) of benzene-H₁², b.p. 78.5°, n_D^{25} 1.498, is collected.

METHOD II



L. H. P. Weldon and C. L. Wilson, *J. Chem. Soc.*, 1946, 235.

A. Procedure (Note 4)

Phenylmagnesium bromide is prepared from 31.2 ml. (0.3 mole) of bromobenzene and 7.2 g. (0.3 g. atom) of magnesium in 154 ml. of sodium-dried ether. The Grignard reagent is then heated at 100° in a vacuum for 48 hours before addition of 5.4 ml. (0.3 mole) of water-H₂² (99.95 atom per cent H²) to the dry reagent which is still under vacuum. The benzene-H₁² and a little ether are collected in a flask cooled with liquid nitrogen, and the residue is treated with a second portion of water-H₂² (5.4 ml.). The total product is washed with hydrochloric acid to remove ether and dried over phosphorus pentoxide. The yield of benzene-H₁² is 40% (Note 5).

B. Notes

1. The preparation of lithium sand is described by Lauer and Noland.
2. This removes most of the bromobenzene and all of the biphenyl.
3. A 24-cm. column with Cannon protruded packing was used.

4. Treatment of the usual Grignard reagent in ether with water- H_2^2 resulted in a product with 29% less deuterium than was expected, according to density measurements. (It is possible that the product was not ether-free.) In view of this result, Weldon and Wilson tried other solvents, including dioxane, methylal and anisole. Finally, dry phenylmagnesium bromide was treated with water- H_2^2 .

5. The density of the product indicated a deuterium content of 17.0 atom per cent, and the density of the combustion water indicated 16.5 atom per cent, compared to a theoretical value of 16.7.

C. Other Preparations

Benzene- H_1^2 has been prepared by the action of water- H_2^2 on phenylmagnesium bromide^{1,3} and by the hypophosphorous acid reduction of benzenediazonium chloride in water- H_2^2 .⁴ Benzene- H_1^2 has also been prepared by heating calcium benzoate with calcium hydroxide- H_2^2 ^{5,6} and with sodium hydroxide- H_2^2 .⁶ In the latter reference, it was shown that exchange also occurs, resulting in a random distribution of deuterium.

In a study⁷ of the deuterium isotope effect in methanolysis of organometallic compounds, benzene- H_1^2 has been prepared by the reaction of methanol- H^2 with phenylmagnesium bromide and with phenyllithium. The isotope effects, k_H/k_{H^2} , in the two reactions were, respectively, 0.96-0.97 and 0.89-0.91.

Benzene- H_1^2 , m.p. 5.5° (22.5 g., 35.6%), has been prepared⁸ by a modification of Method II which avoids working with solvent-free phenylmagnesium bromide.

¹K. Graupner and E. R. S. Winter, J. Chem. Soc., 1952, 1145.

²W. R. Angus, C. R. Bailey, J. L. Gleave, A. H. Leckie, C. G. Raisin, C. L. Wilson and C. K. Ingold, Nature, 135, 1033 (1935).

³W. G. Dauben, G. C. Pimentel and C. W. Vaughan, Jr., J. Am. Chem. Soc., 77, 2886 (1955).

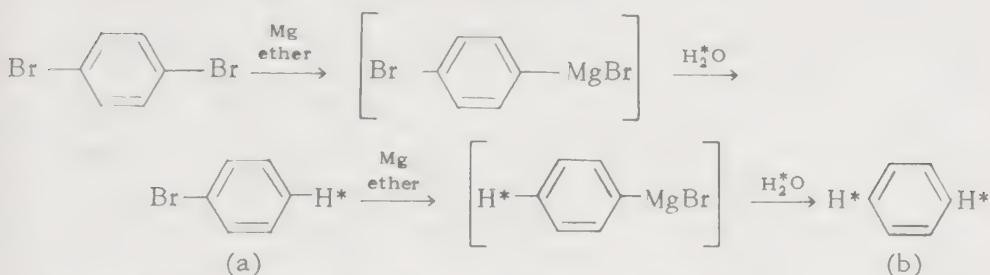
⁴E. R. Alexander and R. E. Burge, Jr., *ibid.*, 72, 3100 (1950).

⁵N. Morita and T. Titani, Bull. Chem. Soc. Japan, 10, 557 (1935).

⁶L. H. P. Weldon and C. L. Wilson, J. Chem. Soc., 1946, 244.

⁷K. B. Wiberg, J. Am. Chem. Soc., 77, 5987 (1955).

⁸D. Bryce-Smith, V. Gold and D. P. N. Satchell, J. Chem. Soc., 1954, 2743.

BENZENE-1,4-H₂²

L. H. P. Weldon and C. L. Wilson, J. Chem. Soc., 1946, 235.

A. Procedure (Note 1)

(a) *1-Bromobenzene-4-H²*. *p*-Phenylenedimagnesium bromide is prepared from 0.8 mole of 1,4-dibromobenzene and 1.6 g. atom of magnesium in 500 ml. of ether. After the addition of water-H₂² to the Grignard reagent, the product is fractionated to obtain 25 g. of 1-bromobenzene-4-H² (Note 2) and 50 ml. of benzene-1,4-H₂², which, after 10 washes with concentrated hydrochloric acid to remove ether, is reduced to 35 ml. (52%).

(b) *Benzene-1,4-H₂²*. 1-Bromobenzene-4-H², 34.5 g. (0.22 mole) is reacted with 6 g. (0.25 g. atom) of magnesium in 112 ml. of ether. The resulting phenylmagnesium-4-H² bromide solution is treated with 13.8 g. (0.69 mole) of water-H₂². Benzene-1,4-H₂² and ether are removed by distillation and separated by fractionation, and the last traces of ether are removed by washing the product with concentrated hydrochloric acid. The product, m.p. 5.9°, after drying over potassium carbonate and then phosphorus pentoxide, is distilled in vacuum; yield 9 ml. (46%) (Note 3).

B. Notes

1. Weldon and Wilson explored several approaches to the synthesis of benzene-1,4-H₂² in a pure form without dilution by hydrogen. The reaction of water-H₂² with dry *p*-phenylenedimagnesium bromide (see benzene-H₁²) was violent and unsuccessful. They also repeated the work of Langseth and Klit¹ and found hydrogen-H² bromide to be slightly superior to hydrogen-H² chloride in reactions with *p*-phenylenedimagnesium bromide. Benzene-1,4-H₂² was prepared which contained 31.4 atom per cent deuterium (by combustion) compared to the theoretical value of 33.3%. In the method of Langseth and Klit,¹ the introduction of hydrogen-H² halide is begun as soon as the formation of the Grignard reagent is started.

2. The benzene-1,4-H₂² analyzed 29.9 atom per cent deuterium (by combustion) compared to the calculated value of 33.3 atom per cent. The 1-bromobenzene-4-H² was quite pure, according to the analysis, and con-

tained 20.2 atom per cent deuterium (by combustion) compared to the calculated 20.0 atom per cent. This fact made it possible to prepare a more nearly pure benzene-1,4- H_2^2 by repeating the Grignard reaction with the 1-bromobenzene-4- H^2 .

3. In the reaction of the dimagnesium compound with water- H_2^2 , Weldon and Wilson took great care to exclude ordinary water vapor and, therefore, offer an alternative explanation for the appearance of hydrogen in place of deuterium in the product. The difference in isotopic composition between the benzene-1,4- H_2^2 and the 1-bromobenzene-4- H^2 shows that, towards some source of hydrogen, the *p*-phenylenedimagnesium bromide is much more reactive than *p*-bromophenylmagnesium bromide. With the dimagnesium compound, in particular, secondary reactions take place by a radical mechanism. There is considerable evidence that some of the reactions of Grignard compounds, in general, proceed by a radical mechanism. Reducing behavior is especially likely to consist in the transfer of single electrons, and it is known that, in the reduction of carbonyl compounds by Grignard reagents, pinacols are among the products.² Hey and Waters³ have shown that it is characteristic of free-radical centers (including aryl), when formed in hydrocarbon, or other hydrogen-containing organic solvents, to abstract a hydrogen atom from the solvent. It may plausibly be supposed that towards some highly reactive Grignard compounds, such as the dimagnesium compound, under suitable conditions the solvent ether may yield hydrogen in this way.

C. Other Preparations

Langseth and Klit¹ have prepared benzene-1,2- H_2^2 , benzene-1,3- H_2^2 and benzene-1,4- H_2^2 by the reaction of hydrogen- H^2 chloride with the corresponding Grignard reagent. The same three H_2^2 -benzenes have been prepared by the reaction of water- H_2^2 with the Grignard reagents.⁴ Benzene-1,2- H_2^2 has also been prepared⁵ by heating a mixture of calcium oxide, calcium hydroxide- H_2^2 and calcium phthalate.

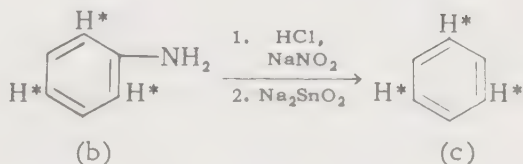
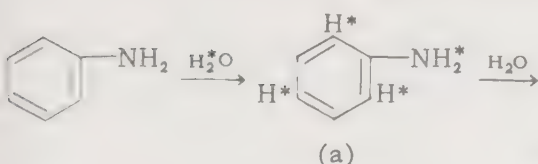
¹A. Langseth and A. Klit, Kgl. Danske Videnskab, Selskab, Math- fys. Medd. 15, No. 13, 22 pp. (1937); Chem. Abstracts, 32, 2516 (1938).

²E. R. Boyd and H. H. Hatt, J. Chem. Soc., 1927, 898.

³D. H. Hey and W. A. Waters, Chem. Revs. 21, 169 (1937).

⁴O. Redlich and W. Stricks, Monatsh, 67, 213 (1936); *ibid.*, 68, 374 (1937).

⁵O. Redlich and W. Stricks, *ibid.*, 68, 42 (1937).

BENZENE-1,3,5- H_3^2 

A. P. Best and C. L. Wilson, J. Chem. Soc., 1946, 239.

A. Procedure (Note 1)

(a) *Aniline-N,N,2,4,6- H_5^2* . Aniline hydrochloride, 13 g., is heated with 20 g. of water- H_2^2 in sealed Pyrex bulbs, immersed in boiling water for 24 hours (Note 2). Using high-vacuum technique, the exchanged water is removed by distillation and replaced by a fresh sample of water- H_2^2 . The salt is treated six times in this manner, the first five times with water containing 99.2 atom per cent and the last time with water containing 99.95 atom per cent deuterium.

(b) *Aniline-2,4,6- H_3^2* . The amino-hydrogens- H_2^2 are removed by exchange, at room temperature, with five successive portions (30 ml. each) of ordinary water.

(c) *Benzene-1,3,5- H_3^2* . The aniline-2,4,6- H_3^2 is dissolved in 60 ml. of water, 20 ml. of concentrated hydrochloric acid is added, and the mixture is cooled to 0° . Sodium nitrite, about 8 g., is added slowly, until a positive reaction for nitrous acid is obtained (starch-iodide), with the temperature maintained below 5° . After 30 minutes in darkness, the solution is added to a solution of 15 g. of sodium hydroxide in 60 ml. of water, and the whole is cooled to -8° . This solution is then added to a solution of sodium stannite (Note 3) in a 500-ml. flask fitted with a reflux condenser and cooled in ice (Note 4). As the mixture warms to room temperature, an oily layer separates, and the benzene is removed by steam-distillation and collected in a receiver cooled in ice. The product is distilled, *in vacuo*, into a bulb containing phosphorus pentoxide and then into a storage bulb. The yield of benzene-1,3,5- H_3^2 is about 65% (Note 5).

B. Notes

1. Weldon and Wilson¹ have made the observation that the modified Grignard method of Langseth and Klit,² for the preparation of H^2 -benzenes,

does not give isotopically pure products; i.e., the atom per cent deuterium in the product does not correspond to the atom per cent deuterium in the hydrogen- H^2 halide used in the reaction. Best and Wilson employed, therefore, the exchange of deuterium between water- H_2^2 and aniline, first obtaining aniline-1,3,5- H_3^2 , which is deaminated to benzene-1,3,5- H_3^2 . Ingold, Raisin and Wilson³ have shown that hydrogen exchange between acids (proton or proton- H^2 donors) and the aromatic nucleus of benzene derivatives shows all the characteristics of an electrophilic substitution process; it is facilitated or retarded, and therefore oriented, in just the same way as are other typical electrophilic aromatic substitutions, such as nitration. It was also demonstrated⁴ that, in the reaction between aniline hydrochloride and water- H_2^2 , the *ortho*- and *para*-positions of the aromatic molecule are the only nuclear positions which participate in the hydrogen exchange.

2. It was established in a preliminary study that equilibrium is attained in 24 hours, under the experimental conditions.

3. The stannite solution is prepared by adding 30% sodium hydroxide slowly to a solution of 30 g. of stannous chloride in 75 ml. of water until the precipitate just redissolves.

4. It was established experimentally that deamination of H^2 -aniline did not affect the nuclear hydrogen- H^2 atoms.

5. Three independent preparations of benzene-1,3,5- H_3^2 by this method gave products having d_{25}^{25} of 0.91100, 0.91113 and 0.91116, respectively. Assuming the linear relationship between the density of benzene and the deuterium content, discussed by Weldon and Wilson,¹ by the use of their values for the densities of benzene and benzene- H_3^2 , the density of pure benzene-1,3,5- H_3^2 is calculated to be 0.91103. The above three samples therefore respectively contain 49.96, 50.15 and 50.18 atom per cent of deuterium in their hydrogen. For the first of these, the combustion method of analysis gave 49.82 atom per cent of deuterium. According to spectroscopic evidence, all three samples were pure benzene-1,3,5- H_3^2 .

Other Methods

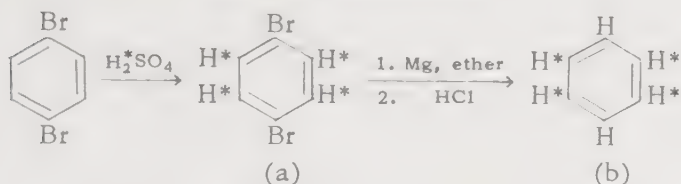
Benzene-1,3,5- H_3^2 has been prepared² by a modified Grignard procedure from 1,3,5-tribromobenzene.

¹L. H. P. Weldon and C. L. Wilson, J. Chem. Soc., 1946, 235.

²A. Langseth and A. Klit, Kgl. Danske Videnskab. Selskab. Math.-fys. Medd., 15, No. 13, (1937).

³C. K. Ingold, C. G. Raisin and C. L. Wilson, J. Chem. Soc., 1936, 1637.

⁴A. P. Best and C. L. Wilson, J. Chem. Soc., 1938, 28.

BENZENE-1,2,4,6-H₄²

A. P. Best and C. L. Wilson, J. Chem. Soc., 1946, 239.

A. Procedure

(a) *1,4-Dibromobenzene-H₄²*. Purified 1,4-dibromobenzene (Note 1), 122.6 g., is divided into three equal portions and shaken in sealed Pyrex bulbs with aqueous sulfuric acid-H₂², at 107° for 30 hours (Note 2). The acid mixture is 88.5 mole per cent sulfuric acid-H₂² and 11.5 mole per cent water-H₂² (Note 3). The quantity of the acid used is sufficient to provide three times as many hydrogen atoms as the number present in the organic compound. After each reaction period, the flasks are cooled, and the acid layer is removed and replaced with fresh acid. Each sample of dibromobenzene is thus treated 5 times (Note 4). The product is dissolved in carbon tetrachloride, washed with sodium carbonate solution and water, and then distilled at ordinary pressure. The yield of 1,4-dibromobenzene-H₄² is 78.6 g., m.p. 87.7–88.4°.

(b) *Benzene-1,2,4,5-H₄²*. 1,4-Dibromobenzene-H₄², 50 g., is reacted with 9 g. of magnesium in 140 ml. of ether. The resulting Grignard reagent is cooled in ice and decomposed by the addition of concentrated hydrochloric acid. The ether layer is washed with sodium carbonate solution and water, dried over anhydrous potassium carbonate and fractionated. This gives a benzene fraction containing a little ether, a benzene-1,2,4,5-H₄² fraction, and a residue, 3.4 g., of 1,4-dibromobenzene-H₄². Residual ether is removed from the benzene-1,2,4,5-H₄² by repeated treatment with perchloric acid (Note 5). The final sample of benzene-1,2,4,5-H₄², 9.49 g., is distilled through a column to ensure elimination of bromo-compounds, dried over phosphorus pentoxide and redistilled (Note 6).

B. Notes

1. The material used in this work was twice recrystallized from alcohol and distilled at ordinary pressure, m.p. 88.1–88.4°.

2. Preliminary experiments showed that conditions which promote hydrogen exchange between benzene and sulfuric acid-H₂², e.g., shaking benzene at room temperature with aqueous sulfuric acid of more than 50 mole per cent sulfuric acid, were useless for 1,4-dibromobenzene, prob-

ably because of the deactivating effect of the bromine atoms towards an electrophilic substituting agent. When the carbon tetrachloride used as solvent was omitted and the fused 1,4-dibromobenzene was shaken with sulfuric acid at 107° , exchange occurred fairly readily. It was then established that it is possible to obtain complete exchange (to equilibrium) without excessive sulfonation, by the use of a mixture of 88.5 mole per cent sulfuric acid and 11.5 mole per cent of water.

3. For the preparation of pure 1,4-dibromobenzene- H_4^2 , the sulfuric acid- H_2^2 is made from sulfur trioxide and pure water- H_2^2 .

4. The first 3 treatments employed acid containing 99.2 atom per cent of deuterium in its hydrogen and raised the deuterium content of the hydrogen of the product to 97.0 atom per cent. The last 2 treatments, which employed acid containing 99.95 atom per cent of deuterium, increased the deuterium content of the hydrogen of the dibromo-compound to 99.45%.

5. It was found, by experiment, that perchloric acid is superior to concentrated hydrochloric or to 70% (by weight) sulfuric acid for this purpose. None of the three reagents caused any isotopic exchange in benzene under the conditions employed. The removal of ether is judged to be complete when the last used portion of the acid no longer evolves ether vapor upon heating under vacuum. To ensure complete removal of ether, the benzene is treated several times more.

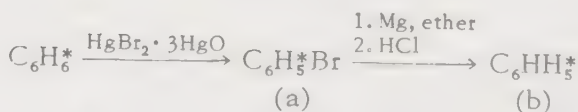
6. The density of the product, d_{25}^{25} 0.92274, corresponded exactly with the theoretical value for 66.67 atom per cent deuterium. Analysis by combustion indicated 66.04 atom per cent deuterium.

C. Other Preparations

Benzene-1,2,4,5- H_4^2 has been prepared by a modified Grignard procedure employing 2,4,5-tribromo-1-iodobenzene.¹

¹A. Langseth and A. Klit, Kgl. Danske Videnskab. Selskab. Math.-fys. Medd., 15, No. 13, (1937).

BENZENE- H_5^2



A. P. Best and C. L. Wilson, J. Chem. Soc., 1946, 239.

A. Procedure

(a) *Bromobenzene- H_5^2* . A solution of mercuric oxybromide is prepared from 53 g. of bromine and 160 g. of mercuric oxide.¹ Together with 8 g.

of benzene- H_6^2 , the solution is shaken in a stoppered bottle for 16 hours. The lower layer is separated and dissolved in ether, and the resulting solution is shaken with sodium carbonate solution to remove mercuric oxybromide. The ethereal bromobenzene solution is then dried and distilled through a column (Note 1). In this way, the crude bromobenzene- H_5^2 is separated into 1.3 g. of benzene- H_6^2 , 8 g. of bromobenzene- H_5^2 , n_D^{20} 1.5600, b.p. 154-155° (761 mm.), and a small amount of crystalline 1,4-dibromobenzene- H_4^2 .

(b) *Benzene- H_5^2* . Magnesium, 1.5 g., and a few crystals of iodine are heated in a 500-ml. flask fitted with a condenser, a dropping funnel and a gas inlet tube. When activation of the magnesium is complete, the air is displaced by dry, oxygen-free nitrogen, and 5 ml. of a solution of bromobenzene- H_5^2 , 7.5 g. in 30 ml. of ether, is added together with 45 ml. of additional ether. When reaction begins, the remainder of the solution of bromobenzene is added slowly, and the reaction is completed by gentle refluxing. The Grignard compound is then decomposed by the cautious addition of concentrated hydrochloric acid, and the ether layer is separated, washed, dried and distilled through a column. The traces of ether remaining in the product are removed by means of perchloric acid (see benzene-1,2,4,5- H_4^2). The final product, m.p. 6.2°, weighs 1.7 g. (Note 2).

B. Notes

1. The chief impurities present, at this stage, in the bromobenzene- H_5^2 were benzene- H_6^2 and 1,4-dibromobenzene- H_4^2 ; their removal by fractionation was followed by determination of refractive index and density. These properties were shown, by preliminary experiments with ordinary materials, to be sensitive indicators of benzene and dibromobenzene respectively.

2. By the combustion method of analysis, the product contained 83.1 atom per cent deuterium compared to the theoretical value of 83.3 atom per cent.

C. Other Preparations

Benzene- H_5^2 has been prepared by the modified Grignard procedure of Langseth and Klit² in 31.4% yield.

Bromobenzene- H_5^2 has been prepared in 83.5% yield by the bromination of benzene- H_6^2 in the presence of fresh iron turnings.³

¹J. Houben, *Die Methoden der Organischen Chemie*, Vol. 3, G. Thieme, Leipzig, 1930, p. 1160.

²A. Langseth and A. Klit, Kgl. Danske Videnskab. Selskab. Math-fys. Medd., 15, No. 13, 7 (1937).

³H. Erlenmeyer, H. Lobeck and A. Epprecht, *Helv. Chim. Acta*, 19, 793 (1936).

BENZENE-H₆²

METHOD I

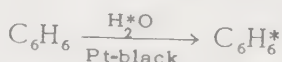


C. K. Ingold, C. G. Raisin and C. L. Wilson, J. Chem. Soc., 1936, 915.

A. Procedure (Note 1)

Pure, thiophene-free benzene is thoroughly dried with phosphorus pentoxide and distilled. The benzene is then added to the sulfuric acid-H₂² reagent (Note 2), by distillation under vacuum, and the mixture is shaken for 3-4 days. The benzene is transferred by vacuum-distillation to a fresh portion of the acid-H₂² and again shaken. This operation is repeated 4 times, and the benzene-H₆² is transferred to a bulb containing barium oxide previously heated for 1 hour at red heat in a high vacuum. After contact for 12 hours with this reagent, the product is transferred into a bulb containing phosphorus pentoxide, from which, 15 minutes later, it is distilled into a storage bulb (Note 3). This procedure gives benzene-H₆² of not less than 99.8 atom per cent deuterium; m.p. 6.8°; b.p. 79.3°; d₂₅²⁵ 0.94543 (Note 4).

METHOD II



L. C. Leitch, Can. J. Chem., 32, 813 (1954).

A. Procedure

A mixture of 5 ml. of benzene (Note 5), 10.0 ml. of water-H₂² and 0.3 g. of platinum-black (Note 6) is sealed in a tube equipped with a break-seal. The tube is heated in a rocking furnace for 12 hours at 110°. After the heating period, the tube is attached to a vacuum manifold and opened with a magnetic hammer. The product is fractionated *in vacuo* and then distilled through a U-tube containing Drierite into a cold trap. Benzene-H₆² is recovered nearly quantitatively. After a small sample is removed for analysis, the remainder is distilled into another reaction tube containing platinum-black and fresh water-H₂². The mixture is heated, and the product is isolated as before. This procedure is repeated twice more to obtain a product containing 95.24% of benzene-H₆² and 3.96% of benzene-H₅² (Note 7).

B. Notes

1. Although benzene- H_6^2 has been prepared on a number of occasions by different methods (see Other Preparations), Ingold, Raisin and Wilson were the first to prepare a nearly isotopically pure product (99.8 atom per cent deuterium in the hydrogen).

2. The sulfuric acid- H_2^2 is prepared from pure sulfur trioxide and water- H_2^2 (99.95 atom per cent deuterium). The benzene is added to the sulfuric acid- H_2^2 (51-52 mole per cent) in the proportion of 1/3 mole of benzene to 3 moles of water- H_2^2 in the diluted acid.

3. All transfers of benzene were made by distillation in an oil-pump vacuum, the vacuum line being provided in advance with the necessary number of bulbs and constrictions for sealing. Liquid air was used to prevent access of oil vapors from the pump and to cool the benzene during the transfers and while making seals.

4. The density of benzene- H_6^2 , corrected for the small amount of residual protium, is d_{25}^{25} 0.9456 or d_4^{25} 0.9429.

5. The benzene was dried by distillation from sodium.

6. The catalyst was prepared by reducing platinum oxide with hydrogen- H_2^2 .

7. This procedure has advantages over Method I in that the reaction time is shortened and the tedious preparation of sulfuric acid- H_2^2 is avoided.

C. Other Preparations

Benzene- H_6^2 has been prepared by the polymerization of acetylene- H_2^{1-4} but is difficult to purify because of numerous other products. Clemo and Robson³ isolated toluene- H_8^2 , indene- H_8^2 , naphthalene- H_8^2 , fluorene- H_{10}^2 and pyrene- H_{10}^2 , in addition to benzene- H_6^2 from the polymerization product. Klit and Langseth⁵ obtained benzene- H_6^2 , with 98 atom per cent deuterium from benzene, aluminum chloride and hydrogen- H^2 chloride. Benzene- H_6^2 has been prepared by the nickel-catalyzed exchange between benzene and water- H_2^2 .⁶⁻⁸ Erlenmeyer and Lobeck⁹ prepared benzene with 93.2% deuterium by the decarboxylation of calcium mellitate with calcium hydroxide- H_2^2 .

Dixon¹⁰ prepared benzene- H_6^2 by a procedure essentially the same as that of Ingold. Physical properties recorded for benzene- H_6^2 (100% deuterium) were: d_{20}^{20} 0.9494; n_D^{20} 1.49911; molar refraction at 20°, 26.03; molar volume at 20°, 88.63.

¹J. W. Murray, C. F. Squire and D. H. Andrews, J. Chem. Phys., 2, 714 (1934).

²G. R. Clemo and A. McQuillen, J. Chem. Soc., 1935, 851.

³G. R. Clemo and A. C. Robson, *ibid.*, 1939, 429.

⁴J. Goubeau, H. Luther, K. Feldmann and G. Brandes, Ber., 86, 214 (1953).

⁵A. Klit and A. Langseth, *Nature*, 135, 956 (1935); *Z. physik. Chem.*, A176, 65 (1936).

⁶P. I. Bownan, W. S. Benedict and H. S. Taylor, *J. Am. Chem. Soc.*, 57, 960 (1935).

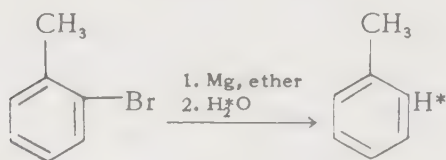
⁷N. R. Trenner, R. W. Walker, B. Arison and R. P. Buks, *Anal. Chem.*, 21, 285 (1949).

⁸J. Horiuti and M. Polanyi, *Nature*, 134, 377 (1934).

⁹H. Erlenmeyer and H. Lobeck, *Helv. Chim. Acta*, 18, 1464 (1935).

¹⁰J. A. Dixon and R. W. Schiessler, *J. Am. Chem. Soc.*, 76, 2198 (1954).

TOLUENE-2-H²



J. Turkevich, H. A. McKenzie, L. Friedman and R. Spurr, *J. Am. Chem. Soc.*, 71, 4045 (1949).

A. Procedure

Toluene-2-H² is prepared by the hydrolysis of *o*-tolylmagnesium bromide according to an adaptation of the method of Weldon and Wilson¹ (Note 1) for the preparation of benzene-H₁². After the formation of the Grignard reagent, most of the ether is distilled off using a vacuum system (Note 2). The reaction flask is cooled in a Dry Ice-acetone bath, water-H₂² is added dropwise, and the mixture is allowed to warm slowly, overnight. After the reaction mixture is shaken vigorously and set aside for at least 24 hours, the product and remaining ether are removed from the flask by vacuum distillation to a trap cooled with liquid air. The contents of the trap are then twice redistilled to obtain a fraction boiling at 110°.

Toluene-3-H², toluene-4-H² and toluene- α -H₁² are also prepared by the same procedure using the corresponding tolylmagnesium bromide (Note 3).

B. Notes

1. In the procedure of Weldon and Wilson,¹ the Grignard reagent is prepared, freed of ether and hydrolyzed without opening the apparatus. The latter procedure eliminates possible exposure of the reagent to atmospheric moisture.

2. It was found preferable not to remove the ether completely since complete removal would make it extremely difficult to effect the hydrolysis with water-H₂².

3. The mass spectra of the H^2 -toluenes indicated that there was less than 0.2% of H_2^2 -compound present in each sample and, at most, 5% non-deuterated toluene.

C. Other Preparations

Toluene-2- H^2 and toluene-3- H^2 have been prepared in 85% and 45% yields, respectively, by reaction of the corresponding Grignard reagent, in butyl ether, with sulfuric acid- H_2^2 .

Toluene- α - H_1^2 , toluene-2- H^2 , toluene-3- H^2 and toluene-4- H^2 have been prepared by hydrolysis of the appropriate Grignard reagent with water- H_2^2 .³⁻⁶

In a study of the deuterium isotope effect,⁷ toluene- α - H_1^2 has been prepared by reaction of methanol- H^2 and benzylmagnesium chloride; the isotope effect, $k_{\text{H}}/k_{\text{H}^2}$, was 0.93-0.99.

Toluene- α - H_1^2 has also been prepared⁸ by the reaction of excess hydrogen- H^2 chloride with benzylmagnesium chloride.

¹L. H. P. Weldon and C. L. Wilson, J. Chem. Soc., 1946, 235.

²C. D. Hurd and J. Azorlosa, J. Am. Chem. Soc., 73, 37 (1951).

³A. R. Choppin and C. H. Smith, *ibid.*, 70, 577 (1948).

⁴C. H. Smith, A. R. Choppin and O. A. Nance, *ibid.*, 72, 3260 (1950).

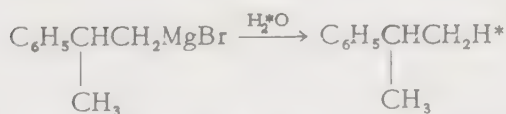
⁵I. I. Kukhtenko, Doklady Akad. Nauk S.S.S.R., 92, 77 (1953).

⁶D. Bryce-Smith, V. Gold and D. P. N. Satchell, J. Chem. Soc., 1954, 2743.

⁷K. B. Wiberg, J. Am. Chem. Soc., 77, 5987 (1955).

⁸H. C. Brown and G. A. Russell, *ibid.*, 74, 3995 (1952).

CUMENE- β - H_1^2 [(1-Methylethyl-2- H_1^2)benzene]



R. L. Burwell, Jr., F. Hummel and E. S. Wallis, J. Org. Chem., 1, 332 (1937).

A. Procedure (Note 1)

A 20-ml. bulb, containing 0.4 g. of ether-washed magnesium, is sealed to the vacuum apparatus, which is then evacuated and heated with a flame to eliminate adsorbed gases. After several hours of evacuation, the bulb is cooled in liquid nitrogen, and dry nitrogen (Note 2) is admitted to the apparatus to a pressure 20 mm. in excess of atmospheric. The seal over the bulb is heated and blown out with the nitrogen pressure. A very small crystal of iodine, 1.75 ml. of D- β -bromocumene and 10 ml. of dry ether are added, and the opening is resealed. Water cooled to 0° is run through the vertical condenser above the bulb, and the re-

action mixture is warmed to room temperature. After 10 minutes of spontaneous reaction, the ether is refluxed for 1 hour (Note 3).

The reaction bulb and a second 20-ml. bulb attached to the manifold are cooled with liquid nitrogen, and the seal over the second bulb is blown out with dry nitrogen; 1.0 ml. of water- H_2^2 (99.5%) is placed in the bulb, and the opening is resealed. The apparatus is evacuated, the water- H_2^2 is distilled into the Grignard reagent, nitrogen is admitted to the apparatus, and the mixture is warmed to room temperature. After the reaction mixture is refluxed for two hours, the volatile materials are vacuum-distilled into the second bulb. Nitrogen is admitted to the apparatus, the reaction flask is removed, and a new bulb containing phosphorus pentoxide is sealed in its place. The product is vacuum-distilled onto the anhydride and then back into the second bulb (Note 4). From 1.08 g. of the ether solution, distilled in a micro apparatus, is obtained 0.64 g. of cumene- $\beta\text{-H}_1^2$, b.p. 151–152° (Note 5).

B. Notes

1. This entire synthesis was carried out in a small all-glass vacuum apparatus with one stopcock. Introduction of reagents was made by blowing out prepared entrances with nitrogen pressure and then re-sealing the openings. In this way, contact with organic hydrogen compounds and atmospheric water vapor was eliminated.

2. Tank nitrogen was passed through a tube containing copper wool at 470°, thence through soda lime, calcium chloride, a trap cooled with Dry Ice-toluene and a trap cooled with liquid air.

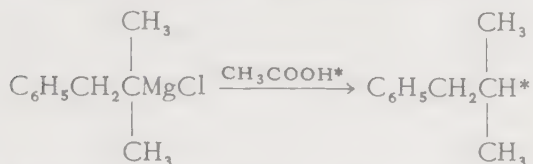
3. The apparatus was vented to the atmosphere through a manometer-type mercury valve.

4. A sample of the ether solution did not display an observable rotation at 25° in a 1-dm. tube.

5. The rotation of the undiluted product was then determined: $[\alpha]_{\text{D}}^{25} + 0.019^\circ$, 1-dm. tube, average of 11 readings with maximum deviation from the mean 0.011° . Similar experiments carried out on β -bromocumene, $[\alpha]_{\text{D}} - 1.0^\circ$, with water- H_2^2 gave a cumene- $\beta\text{-H}_1^2$ with no observable rotation. Apparently the maximum rotation of compounds of this type is very small. The theoretical considerations of Boys² predict a small rotation for compounds the asymmetry of which is due to the differences between deuterium and hydrogen.

¹J. B. Cohen, J. Marshall and H. E. Woodman, J. Chem. Soc., 107, 887 (1915).

²S. F. Boys, Proc. Roy. Soc. (London), A144, 655 (1934).

(2-METHYLPROPYL-2-H²)BENZENE

W. G. Brown, C. J. Mighton and M. Senkus, J. Org. Chem., 3, 62 (1939).

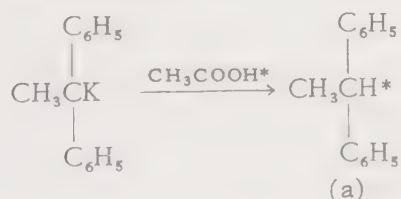
A. Procedure

The Grignard reagent is formed from 7 g. of magnesium and 50 g. of (2-chloro-2-methylpropyl)benzene (Note 1) at 50°, using a drop of methyl iodide to start the reaction (Note 2). The halide is added over a period of 3 hours, after which stirring is continued for an additional hour. A solution of 5.9 g. of acetic acid-H² in 100 ml. of ether is then added slowly with stirring. The reaction mixture is filtered, and the filtrate is shaken successively with small amounts of sodium carbonate and silver nitrate solutions. After removal of the ether, the product is fractionated at atmospheric pressure, b.p. 170.5–171.5°.

B. Notes

1. The chloro compound was prepared by saturating α,α -dimethylphenethyl alcohol at 0° with dry hydrogen chloride and was purified by distillation under reduced pressure, b.p. 86–89° (10 mm.).

2. It was found desirable to begin with a relatively concentrated solution of the halide in ether, about 1:1, and to dilute the halide further during the addition so that the final volume was about 1 liter; otherwise the mixture became too viscous for smooth reaction.

1,1-DIPHENYLETHANE-1-H²

W. G. Brown, C. J. Mighton and M. Senkus, J. Org. Chem., 3, 62 (1939).

A. Procedure

(a) *1,1-Diphenylethane-1-H²* (Note 1). The successful preparation of this compound is accomplished by means of Ziegler's ether cleavage

method¹ for the preparation of alkali metal compounds. The potassium compound is prepared according to the procedure of Ziegler and Schnell² (Note 2). Methyl 1,1-diphenylethyl ether, 30 g., is placed in a nitrogen filled flask together with 300 ml. of anhydrous ether and 10 g. of sodium-potassium alloy (1:5). The flask is closed and shaken mechanically for 24 hours. A solution of 6.85 g. of acetic acid- H^2 in 20 ml. of ethyl ether is then added, whereupon the red color of the mixture is immediately discharged. The material is filtered, ether is removed, and the residual liquid is distilled under reduced pressure. The yield of 1,1-diphenylethane- $1-H^2$, b.p. $136-137^\circ$ (12 mm.), is 22 g.

(b) *Cumene- α - H^2* (Note 3). Methyl α,α -dimethylbenzyl ether, 10 g., is dissolved in 500 ml. of anhydrous ethyl ether and treated with 10 g. of sodium-potassium alloy (1:5) as in the above procedure (Note 4). A solution of 4 g. of acetic acid- H^2 in ether is then added, the mixture is filtered and, after removal of ether, the product is fractionated; b.p. $149-151^\circ$.

(c) *Triphenylmethane- H^2* . Triphenylmethylsodium is prepared according to the method of Schlenk³ (Note 5). An ether solution containing 25 g. of chlorotriphenylmethane is poured quickly into the reaction flask, and ether is added to make a total of 800 ml. To this solution is added 1200 g. of 1% sodium amalgam, and the stoppered flask is shaken mechanically for 2 hours. The equivalent amount of acetic acid- H^2 , prepared from acetic anhydride and water- H_2^2 , is added with stirring, and the ether layer is separated. After evaporation of the ether a yellow residue remains, which, on recrystallization from alcohol, yields 15 g. of triphenylmethane- H^2 , m.p. $91-92^\circ$.

B. Notes

1. Unsuccessful attempts were made to develop a procedure for the preparation of 1,1-diphenylethane- $1-H^2$ from 1-chloro-1,1-diphenylethane *via* the Grignard reagent and by reduction with zinc in acetic acid. The chloro compound was prepared according to the method of Schoepfle and Ryan.⁴ Employing a variety of forms of activated magnesium, no reaction could be obtained at temperatures below 40° . The action of zinc dust in acetic acid resulted in the formation of 1,1-diphenylethylene.

2. An improved procedure is given by Brown, Mighton and Senkus for purifying the methyl 1,1-diphenylethyl ether.

3. As in the case of 1,1-diphenylethane- $1-H^2$, unsuccessful attempts were made to use the Grignard procedure and the zinc-acetic acid method with 2-chloro-2-phenylpropane.

4. Procedures for the preparation and purification of methyl α,α -dimethylbenzyl ether are given by Brown, *et al.*, also.

5. The entire reaction was carried out in a long-necked 1-l. flask with a side-arm which served as an outlet for nitrogen gas and for the introduction of reagents. Commercial nitrogen was used after purification by passage, in the following order, over a copper spiral at 450° , through three gas bubblers containing Fieser's solution,⁵ a bubbler containing sulfuric acid and a series of towers containing potassium hydroxide, calcium chloride and phosphorus pentoxide.

C. Other Preparations

Triphenylmethane- H^2 has been prepared⁶ from chlorotriphenylmethane, zinc dust and acetic acid- H^2 . This was an adaptation of Gomberg's⁷ method for the preparation of triphenylmethane, and is the most convenient method of preparing this hydrocarbon from the chloride, but requires an excess of acetic acid- H^2 . In addition, the Raman spectrum of the product exhibited a number of new $C-H^2$ lines, in addition to that shown by the product prepared from triphenylmethyllsodium. These additional lines were quite probably due to aromatic $C-H^2$ linkages, since they checked fairly well with lines occurring in the Raman spectra of deuterated benzenes.⁸ Hence, it was indicated that some exchange of hydrogen occurred during preparation of the compound.

¹K. Ziegler and F. Thielmann, *Ber.*, 56, 1740 (1923).

²K. Ziegler and B. Schnell, *Ann.* 437, 227 (1924).

³Houben-Weyl, *Die Methoden der organischen Chemie*, Vol. 4, George Thieme, Leipzig, 1923, p. 974.

⁴C. S. Schoepfle and J. D. Ryan, *J. Am. Chem. Soc.*, 52, 4021 (1930).

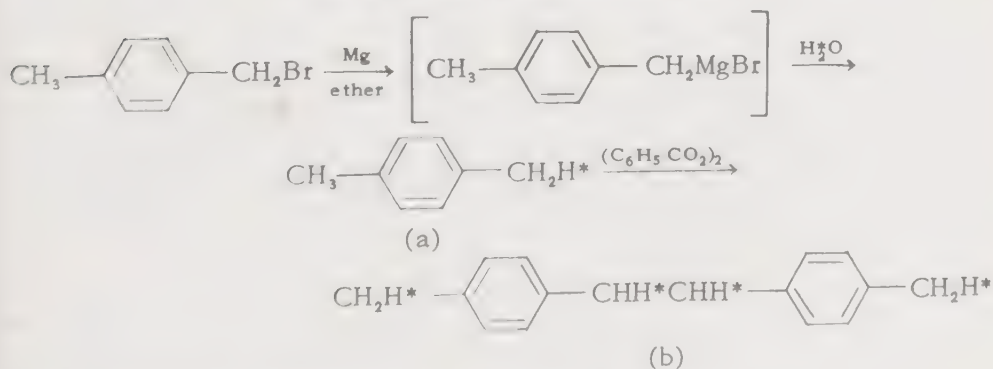
⁵L. F. Fieser, *ibid.*, 46, 2639 (1924).

⁶W. G. Brown, C. J. Mighton and M. Senkus, *J. Org. Chem.*, 3, 62 (1939).

⁷M. Gomberg, *Ber.*, 36, 383 (1903).

⁸R. W. Wood, *J. Chem. Phys.*, 3, 444 (1935).

H^2 -4,4'-DIMETHYLBIBENZYL



J. I. G. Cadogan, V. Gold and D. P. N. Satchell, *J. Chem. Soc.*, 1955, 561.

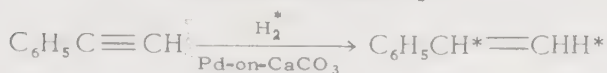
A. Procedure

(a) *p*-Xylene- α - H_1^2 . To the Grignard reagent prepared from 0.23 mole of *p*-methylbenzyl bromide in ether is slowly added dropwise (during 20 minutes) 0.4 mole of water- H_2^2 . The ether solution of *p*-methylbenzylmagnesium bromide is vigorously agitated and kept at its boiling point during the addition. The mixture is stirred for an additional 30 minutes, and solid carbon dioxide is then added to remove unchanged Grignard reagent. The mixture is acidified with 1 *N* hydrochloric acid, and the layers are separated. The ether solution is washed, successively, with water, twice with 2 *N* sodium hydroxide solution and again with water. The solution is dried over calcium chloride, and evaporation of the solvent leaves a pale yellow oil which is distilled through a column packed with glass helices. The fraction boiling at 138° (760 mm.) is refractionated from sodium metal. The yield of *p*-xylene- α - H_1^2 , b.p. 138° (760 mm.), m.p. 13° (Note 1), is 60% (Note 2).

(b) H^2 -4,4'-Dimethylbibenzyl. Benzoyl peroxide, 1.9 g., is added, during 10 minutes, to 20 g. of *p*-xylene- α - H_1^2 kept at 80° in a thermostat. After 72 hours, the decomposition of benzoyl peroxide is considered to be complete, and the excess of *p*-xylene- α - H_1^2 is removed through a helix-packed column. The residual oil is heated under reflux with 30 ml. of 2.5 *N* sodium hydroxide solution for 8 hours (Note 3). The mixture is then extracted with four 20-ml. portions of benzene, and the combined extracts are washed with water and dried over calcium chloride. The benzene is removed by distillation through a 10-inch column, and the residual pale yellow oil is distilled *in vacuo*, giving 1.10 g. of a pale-yellow semi-solid product, b.p. 60-90° (0.15 mm.). The liquid present is removed by filtration through a sintered glass funnel, and the solid residue (0.75 g.) is recrystallized to a constant melting point of 82° from aqueous methanol. The yield of H^2 -4,4'-dimethylbibenzyl (colorless plates) is 0.23 g.

B. Notes

1. The melting point was unchanged by fractional freezing.
2. The product contained 6.95 atom per cent deuterium. Alkaline permanganate oxidation of a portion of the product gave terephthalic acid with 0.47 atom per cent deuterium, indicating the presence of a small amount of deuterium in the nucleus.
3. This was done to hydrolyze the esters present.

STYRENE- α, β -H²

E. R. Bissell, U. S. Atomic Energy Comm. Report, UCRL-4472; Nuclear Sci. Abstr., 9, 4413 (1955).

A. Procedure

To a suspension of 5.0 g. of 2% palladium-on-carbon catalyst¹ in 50 ml. of ether, in a 500-ml. Parr hydrogenation bottle, is added 96.6 g. of vinylbenzene. The reaction mixture is then treated with hydrogen-H₂² until the calculated amount is absorbed (Note 1). The ether solution is freed of catalyst, which is washed with a little ether and reused in the subsequent runs. In all, 7 batches of vinylbenzene are hydrogenated (Note 2). The solvent is removed from the combined ether solution, and the residue is distilled under reduced pressure through a 30-cm. Vigreux column. The yield of styrene- α, β -H₂² is 698.4 g. (74.7%), b.p. 38–41° (15 mm.), from a total of 908.6 g. of vinylbenzene. The center portion of a sample redistilled through a small helix-packed column has the following properties: b.p. 40–41° (15 mm.), n_D^{25} 1.54239, m.p. –34.3° to –30.5°, d_{25}^{25} 0.9178 (Note 3).

B. Notes

1. With the apparatus used, it was not possible to control the hydrogen-H₂² absorption to the degree of precision desirable if (ethyl-1,1,2,2-H₄²)-benzene and unreacted vinylbenzene are to be eliminated from the product.

2. The presence of unreacted vinylbenzene in the product was detectable by polymerization of a small sample of the product in an evacuated, sealed tube at 150° for 24 hours. Even small amounts of vinylbenzene imparted a yellow color to the polystyrene. Vinylbenzene could be removed from the styrene, with some loss of styrene due to polymerization, by dilution of the styrene with dry ether and treatment of this solution with sodium wire at 0°.

3. Nuclear magnetic resonance measurement of the H²:H ratio in the product indicated 24.4 ± 1 atom per cent deuterium. The ultraviolet absorption spectrum was identical with that of normal styrene with the exception that the peak at 2820 Å was shifted to 2825 Å.

C. Other Preparations

Styrene- α -H² has been prepared² by the reduction of acetophenone with lithium aluminum hydride-H₄² and dehydration of the resulting α -methylbenzyl- α -H² alcohol (see styrene- α -H³). The over-all yield was 74.5%

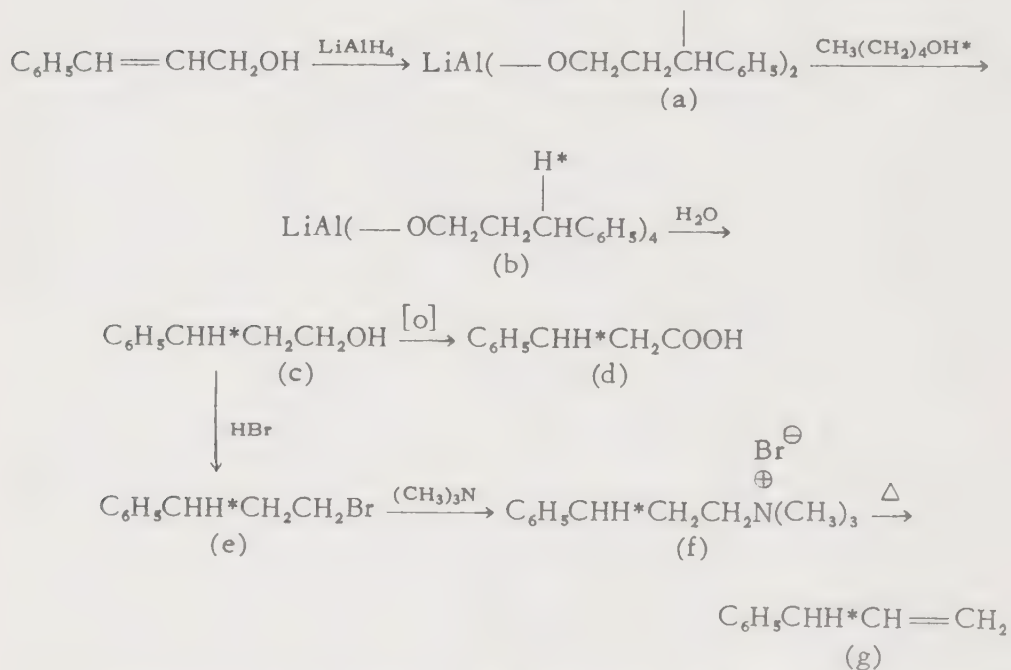
based on acetophenone; the loss occurred mainly in the dehydration step. H^2 -Polystyrene was prepared from this material at 70° with essentially complete conversion.

Styrene- β - H_1^2 has been prepared² from β -bromostyrene, in 50% yield, by hydrolysis of the Grignard reagent with water- H_2^2 . H^2 -Polystyrene was also prepared from this product.

¹M. Busch and H. Stone, Ber., 49, 1063 (1916).

²L. A. Wall and D. W. Brown, J. Polymer Sci., 14, 513 (1954).

ALLYL-1- H_1^2 -BENZENE
(3-Phenylpropene-3- H_1^2)



F. A. Hockstein and W. G. Brown, J. Am. Chem. Soc., 70, 3484 (1948).

A. Procedure

(c) *3-Phenyl-1-propanol-3- H_1^2* . Cinnamyl alcohol, 13.4 g. (0.1 mole), dissolved in 35 ml. of ether is added to a solution of 0.055 mole of lithium aluminum hydride in 40 ml. of ether. After the mixture is refluxed for 5 hours, 12.0 g. (0.136 mole) of pentanol- H^2 is added dropwise (Note 1), causing a marked exothermic reaction (Note 2). The solution is kept at room temperature for 10 hours, and then the hydrolysis is completed by the addition of 70 ml. of 20% sulfuric acid (Note 3). The aqueous layer is twice extracted with ether, the combined ether solutions are

dried over potassium carbonate, and the product is recovered by distillation under reduced pressure; yield 12.3 g. (90%).

To 10.7 g. of the H^2 -alcohol is added 21.8 g. of nonisotopic material, and to ensure normalization of the hydroxyl hydrogen the total material is twice treated with 50-ml. portions of ethanol which is removed by distillation. Finally, the 3-phenyl-1-propanol- $3-H_1^2$ is distilled again under reduced pressure, b.p. $131.5-132^\circ$ (20 mm.) (Note 4).

(d) *Hydrocinnamic- $\beta-H_1^2$ Acid*, (*3-Phenylpropionic- $3-H_1^2$ Acid*). (1) According to the procedure of Guerbet,¹ 5 g. of 3-phenyl-1-propanol- $3-H_1^2$ and 7.2 g. of powdered potassium hydroxide (Note 5) are heated at $245-255^\circ$ for 3 hours. The volume of hydrogen evolved is 1.45 liters (88%), and from the solid residue is obtained 4.9 g. (88%) of 3-phenylpropionic- $3-H_1^2$ acid (Note 6). (2) To 4.0 g. of 3-phenyl-1-propanol- $3-H_1^2$ dissolved in 25 ml. of glacial acetic acid is added gradually 3.80 g. of powdered, anhydrous chromic oxide, with cooling and vigorous stirring of the solution. The yield of hydrocinnamic- $\beta-H_1^2$ acid is 0.55 g. (13%), m.p. 47.5° (Note 7).

(e) (*3-Bromopropyl- $1-H_1^2$*)benzene, (*1-Bromo-3-phenylpropane- $3-H_1^2$*). 3-Phenyl-1-propanol- $3-H_1^2$, 14.1 g., is converted to 1-bromo-3-phenylpropane- $3-H_1^2$ by treatment with hydrobromic acid² at the boiling point for 2 hours (molar ratio 1:2). The product is washed with water, dried and distilled under reduced pressure (Note 8) to obtain 18.9 g. of the bromide.

(f) *Trimethyl-3-phenylpropyl- $3-H_1^2$ -ammonium Bromide*. The 18.9 g. of 1-bromo-3-phenylpropane- $3-H_1^2$ is heated under reflux for 6 hours with 10.8 g. of trimethylamine in 100 ml. of absolute ethanol. Upon concentration of the resulting solution, 17.3 g. of the quaternary bromide is obtained as colorless crystalline plates, m.p. $150-152^\circ$.

(g) *Allyl- $1-H_1^2$ -benzene*, (*3-Phenylpropene- $3-H_1^2$*). Without further purification, the quaternary bromide is heated in a distilling flask at $280-320^\circ$. An ether extract of the distillate, which consists of liquid hydrocarbon and solid trimethylamine hydrobromide, is washed successively with water, dilute hydrochloric acid, sodium bicarbonate solution and again with water. In the fractional distillation of the ether extract, 3.20 g. of allyl- $1-H_1^2$ -benzene is collected in the range $156-158^\circ$, n_D^{20} 1.5104 and 0.70 g. in the range $158-160^\circ$, n_D^{20} 1.5176 (Note 9), which together constitute a yield of 68%. A further fraction (0.55 g.), b.p. $160-167^\circ$, n_D^{20} 1.5295 probably contains some additional allylbenzene as well as some propenylbenzene, n_D^{20} 1.5492 (Note 10).

B. Notes

1. The exact nature of the lithium aluminum complex (a) was not known, but experimental evidence indicated a cyclic structure with aluminum to carbon bonds. Although lithium aluminum hydride normally

does not react with double bonds, there are several examples³ in which reduction of a polar functional group is accompanied by reduction of an adjacent double bond.

2. The solution remains clear but becomes very viscous. During the addition, 400 ml. of hydrogen is evolved (equivalent to about 0.004 mole of excess lithium aluminum hydride). Apparently the pentanol reacts with excess hydride and then attacks the aluminum to carbon bond in the 3-position.

3. This is the hydrolysis of the usual lithium aluminum alkoxide (b).

4. Analysis for deuterium showed 17.2 mole per cent $C_9H_{11}H^2O$.

5. Previously fused at red heat.

6. Analysis of the acid indicated 11.8 mole per cent $C_9H_9H^2O_2$. This result indicated either that 37% of the deuterium originally present in the alcohol was present in the carbinol group or that deuterium present in other positions was lost through exchange with potassium hydroxide.

7. This product contained 17.5 mole per cent $C_9H_9H^2O_2$ corresponding to the original alcohol and indicating that exchange did take place with potassium hydroxide.

8. The boiling point given by Norris, *et al.*² is 128–129° (29 mm.).

9. The literature⁴ gives n_D^{20} 1.5143.

10. In view of the known effect of strong alkali in causing the isomerization of allylbenzene to propenylbenzene,^{4,5} it seemed probable that formation of the latter in the thermal decomposition of phenyltrimethylammonium hydroxide is a consequence of the strong alkaline nature of the material. Hockstein and Brown, therefore, prepared the neutral salts, bromide and iodide, and found that both gave allylbenzene. Analysis of the first fraction for deuterium showed 17.2 mole per cent $C_9H_9H^2$. The data indicate that all of the deuterium was attached to carbon-3 of the original 3-phenyl-1-propanol.

¹M. M. Guerbet, Bull. soc. chim. France [4] 11, 164 (1912).

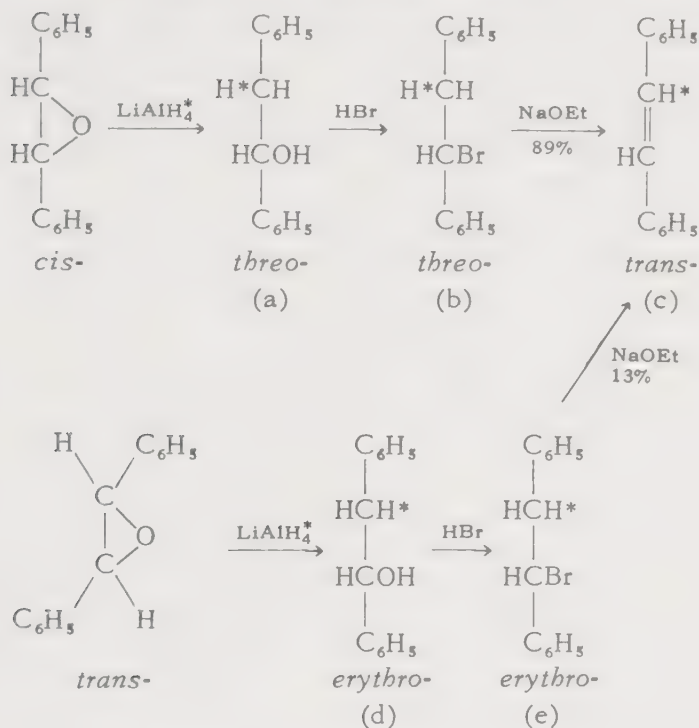
²J. F. Norris, M. Watt and R. Thomas; J. Am. Chem. Soc., 38, 1078 (1916).

³R. F. Nystrom and W. G. Brown, J. Am. Chem. Soc., 69, 1197, 2548 (1947).

⁴M. Tiffeneau, Compt. rend., 139, 482 (1904).

⁵A. Klages, Ber., 39, 2590 (1906).

trans-STILBENE- α -H²
(*trans*-1,2-Diphenylethylene-1-H²)



D. Y. Curtin and D. B. Kellom, J. Am. Chem. Soc., 75, 6011 (1953).

A. Procedure (Note 1)

(a) *threo*-1,2-Diphenylethanol-2-H¹. To 5.03 g. (0.120 mole) of lithium aluminum hydride-H₄¹ (96%) in 200 ml. of dry ether is added 21.0 g. of *cis*- α,α' -epoxybiphenyl in 100 ml. of dry ether during 35 minutes. The grey mixture is stirred at 20° for 15 minutes, at the boiling point under reflux for 30 minutes and again at 20° for 75 minutes. The lithium aluminum alkoxide is hydrolyzed with dilute acid added dropwise. The ether layer, combined with an ether extract of the aqueous phase, is dried over Drierite, and the solvent is removed. The residue is recrystallized from heptane; the yield of white solid melting at 64.4–65.5° is 17.5 g.

(b) *threo*- α -Bromobiphenyl- β -H². A slurry of 1.00 g. (5.05 mmoles) of the *threo*-alcohol in 25 ml. of pentane is cooled in a Dry Ice-acetone bath and anhydrous hydrogen bromide is bubbled through the cold solution for 1 hour. The mixture is allowed to warm to room temperature during 10 minutes while dry nitrogen is swept through the flask. The slightly cloudy solution is poured into water, the organic layer is separated,

washed thoroughly with water, once with 5% sodium carbonate solution and again with water. Evaporation of the solvent, after drying the solution with anhydrous magnesium sulfate, gives 1.18 g. of slightly cloudy liquid. The bromide, purified only by filtration through a small amount of carbon held on a sintered glass funnel (Note 2), is obtained in 70–80% yield as a clear liquid, n_D^{20} 1.6019. Analysis for carbon and hydrogen, and the saponification equivalent indicate the product to be 97% pure (Note 3).

(c) *trans*-Stilbene- α -H². *threo*- α -Bromobibenzyl- β -H₁², 250 mg., is heated under reflux with 20 ml. of 2 *N* sodium ethoxide in absolute ethanol. The yield of *trans*-stilbene- α -H², m.p. 121.8–123°, is 84% with 89% retention of deuterium (Note 4).

(d) *erythro*-1,2-Diphenylethanol-2-H₁². The solid obtained from *trans*- α , α' -epoxybibenzyl, according to the procedure for the *threo*-compound (a) above, is a mixture, m.p. 48–62°, of *erythro*-1,2-diphenylethanol-2-H₁² and unreacted *trans*- α , α' -epoxybibenzyl. Separation is achieved by chromatography on 500 g. of aluminum hydroxide, activated at 350–400° for several hours. The *trans*- α , α' -epoxybibenzyl (2.3 g., m.p. 67.4–68.6°) is removed from the column with hexane. The *erythro*-1,2-diphenylethanol-2-H₁², m.p. 64.4–65.4°, is then recovered by elution with benzene containing a small amount of absolute ethanol. The yield is 13.0 g. (68%).

(e) *erythro*- α -Bromobibenzyl- β -H₁². The *erythro*-form is prepared in the same manner as *threo*-isomer, (b) above (see Note 4).

(f) *erythro*- and *threo*-1,2-Diphenylethyl-2-H₁² 2,4,6-Triethylbenzoate. *erythro*- or *threo*-1,2-Diphenylethanol-2-H₁², 1.81 g. (9.14 mmoles), is heated under reflux with 2.06 g. (9.17 mmoles) of 2,4,6-triethylbenzoyl chloride in 5 ml. of dry benzene for 3 hours. The clear solution is then poured into 40 ml. of hexane; the solution is washed with water, 5% potassium carbonate, again with water, and dried over Drierite. Evaporation of the solution gives 3.13 g. of an oil which slowly crystallizes. Recrystallization of the solid from 10 ml. of heptane gives 2.42 g. (69%) of the triethylbenzoate ester, m.p. 76–78°. Recrystallization from 95% ethanol raises the melting point to 77–77.6°.

(g) *erythro*- and *threo*-1,2-Diphenylethyl-2-H₁² Benzoate. *erythro*- or *threo*-1,2-Diphenylethanol-2-H₁² is treated with benzoyl chloride in pyridine. The yield of benzoate ester is 70%.

(h) *erythro*- and *threo*-1,2-Diphenylethyl-2-H₁² Acetate. The *erythro*- or *threo*-alcohol is treated with acetic anhydride and fused sodium acetate at 100° for 3 hours. The products are colorless oils in 77–87% yields, b.p. 121–123° (0.8 mm), n_D^{15} 1.5500, n_D^{20} 1.5478.

(i) *erythro*- and *threo*- α -Chlorobibenzyl- β -H₁². To 15 ml. of purified thionyl chloride,² cooled in an ice-bath, is added, in small portions, 4.90

g. (0.025 mole) of *erythro*- or *threo*-1,2-diphenylethanol-2- H_1^2 . The clear, yellow solution is warmed at 35–40° for 1.5 hours while a stream of dry nitrogen is bubbled through the solution. Excess thionyl chloride is removed under reduced pressure and the light-brown residue is diluted with 50 ml. of heptane. This solution is washed with water, dilute sodium hydroxide and again with water. The solution, dried over anhydrous magnesium sulfate, is filtered, and the solvent is evaporated in a stream of dry air. The crude, light-yellow product is purified by filtration through charcoal since it decomposes on distillation. The yield is 4.37 g. (82%), n_D^{20} 1.5850 (Note 5).

B. Notes

1. In order to study further the preparation and reactions of diastereoisomers which differ in the positions of hydrogen and deuterium, Curtin and Kellom prepared DL-*erythro*- and DL-*threo*-1,2-diphenylethanol-2- H_1^2 from *trans*- and *cis*- α, α' -epoxybibenzyl, respectively, by treatment with lithium aluminum hydride- H_4^2 . The opening of other epoxides with lithium aluminum hydride has been shown to proceed with inversion of configuration.³ It is believed that lithium aluminum hydride- H_4^2 reactions are highly stereospecific. Such specificity has been observed in the reaction of D-(+)-*trans*-2,3-epoxybutane with lithium aluminum hydride which gave D-(-)-2-butanol of 99% optical purity.⁴

2. The bromide decomposed on distillation, even in vacuum.

3. The bromide can be prepared at 0° in benzene solution with approximately the same yield.

4. Similar treatment of the *erythro*-bromide give an 82% yield of *trans*-stilbene with only 13% retention of the deuterium. Thus, in each case, the bromide is formed from the corresponding alcohol with about 85–90% retention of configuration and 10–15% inversion. When the reaction with hydrogen bromide is carried out at 0° in benzene, there is approximately 75% retention of configuration and 25% inversion.

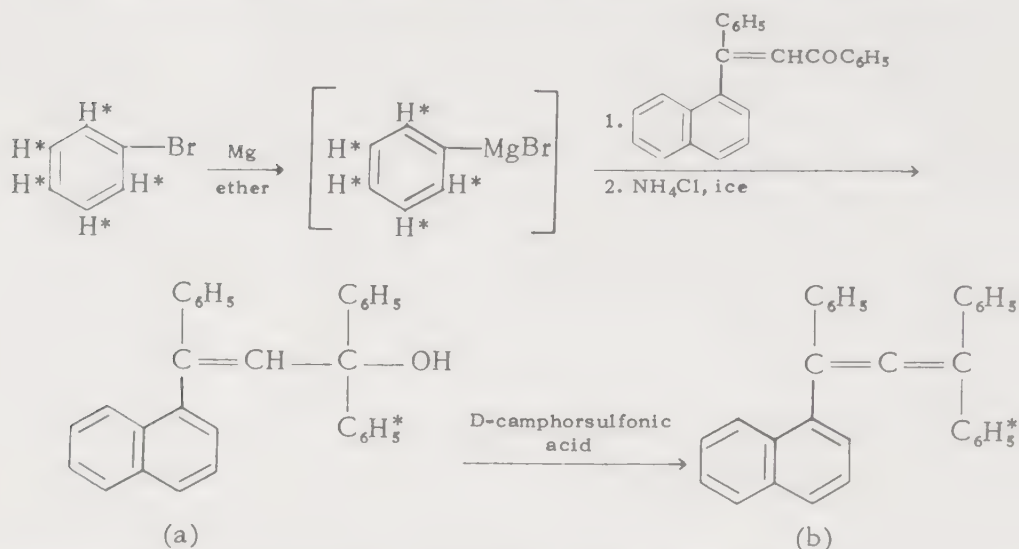
5. The chloro compounds as well as the pairs of isomeric esters (f), (g) and (h) were subjected to the base-catalyzed elimination reaction, also. In addition to the data on these reactions, data on the formation of *trans*-stilbene, from the same intermediates, by thermal elimination, are given in the original work of Curtin and Kellom.

¹Metal Hydrides Inc., Beverly, Mass.

²D. L. Cottle, J. Am. Chem. Soc., 68, 1380 (1946).

³*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 478.

⁴P. J. Leroux and H. J. Lucas, J. Am. Chem. Soc., 73, 41 (1951).

1-(1-NAPHTHYL)-1,3-DIPHENYL-3-PHENYL-H₅²-PROPADIENE

G. R. Clemo, R. Roper and A. C. Robson, J. Chem. Soc., 1939, 431.

A. Procedure

(a) *3-(1-Naphthyl)-1,3-diphenyl-1-phenyl-H₅²-2-propen-1-ol*. A solution of 0.77 g. of β -(1-naphthyl)chalcone¹ in 10 ml. of benzene is run, during 15 minutes, into a refluxing Grignard solution prepared from 0.75 g. of bromobenzene-H₅² and 0.173 g. of magnesium in about 10 ml. of dry ether (Note 1). After 15 minutes at 40–45°, the mixture is diluted with 6 ml. of benzene and hydrolyzed with ice and ammonium chloride. The benzene-ether solution is dried over potassium carbonate, and the solvents are evaporated. A few ml. of petroleum ether is added to the residue, and the alcohol, 0.65 g., crystallizes in clusters, m.p. 147–148°. After recrystallization from benzene-petroleum ether, the product melts at 149–150°.

(b) *1-(1-Naphthyl)-1,3-diphenyl-3-phenyl-H₅²-propadiene*. The above alcohol, 0.5 g., and 5 mg. of D-camphorsulfonic acid are heated under reflux in 25 ml. of benzene for 10 minutes. The product, 0.44 g., is obtained on evaporation of the benzene and addition of petroleum ether; m.p. 100–101° (Note 2).

B. Notes

1. Experiment indicated that in the preparation of 3-(1-naphthyl)-1,1,3-triphenyl-2-propen-1-ol by the method of Maitland and Mills,¹ only two equivalents of phenylmagnesium bromide are required if a solution of the ketone in benzene is added to a boiling solution of the Grignard reagent.

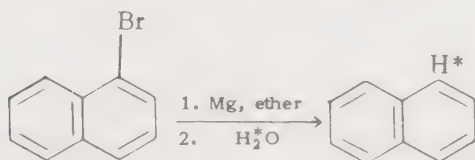
2. The product obtained was not optically active. Although Maitland and Mills,¹ reported the separation of a similar allene into its optical antipodes and observed high rotations for the D- and L-forms, this attempt at asymmetric dehydration by their procedure was unsuccessful.

C. Other Preparations

Clemo, Roper and Robson have obtained 1-(1-naphthyl)-1,3-diphenyl-3-phenyl-H₂²-propadiene, also, from the isomeric 1-(1-naphthyl)-1,3-diphenyl-3-phenyl-H₂²-2-propen-1-ol.

¹P. Maitland and W. H. Mills, J. Chem. Soc., 1936, 987.

NAPHTHALENE-1-H²



J. Goubeau, H. Luther, K. Feldmann and G. Brandes, Ber., 86, 214 (1953).

A. Procedure

(a) *Naphthalene-1-H²*. With stirring, 1.6 g. of water-H₂² is slowly added dropwise to the Grignard reagent prepared from 16 g. of 1-bromonaphthalene and 2 g. of magnesium in 80 ml. of ether. The ether solution is placed in a large sublimation flask, and the ether is distilled off with a water-bath. The sublimation apparatus is assembled, and the last of the ether is removed by warming the flask at 12 mm. Naphthalene-1-H² begins to sublime at 50°, and the majority is sublimed between 80° and 90°. The product, 7.5 g. (75%), is purified through sublimation and by distillation at 0.1 mm.; b.p. 219.4° (752 mm.), m.p. 80.0°.

(b) *Naphthalene-2-H²*. From 16 g. of 2-bromonaphthalene, treated as described above, is obtained 6.9 g. (69%) of naphthalene-2-H², which after purification has: m.p. 80.1° and b.p. 219.2° (755 mm.).

(c) *1-Methylnaphthalene-4-H²*. The Grignard reagent, prepared from 22 g. of 1-bromo-4-methylnaphthalene (Note 1) and 3 g. of magnesium turnings in 80 ml. of ether, is decomposed with 2 g. of water-H₂² at -20°. Ether is removed from the ether extract, and the residue is distilled. The yield of 1-methylnaphthalene-4-H², b.p. 123° (12 mm.), is 6 g. (42%); d_4^{24} 1.0144, n_D^{20} 1.60587.

(d) *1-Ethyl-naphthalene-4-H²*. 4-Ethyl-1-naphthylmagnesium bromide is prepared from 40 g. of 1-bromo-4-ethylnaphthalene (Note 2) and 5 g. of

magnesium turnings in 150 ml. of ether. Treatment of the Grignard reagent with water- H_2^2 yields 14 g. (52%) of 1-ethylnaphthalene-4- H^2 , b.p. 95° (3 mm.), n_D^{20} 1.61651, d_4^{20} 1.0283.

(e) 2-Methylnaphthalene-1- H^2 . Treatment of 22 g. of 1-bromo-2-methylnaphthalene (Note 3), as described for the preparation of 1-methylnaphthalene-4- H^2 , yields 7.5 g. (52%) of 2-methylnaphthalene-1- H^2 ; b.p. 95° (3 mm.), n_D^{20} 1.61651, d_4^{20} 1.0283.

(f) 2-Ethylnaphthalene-1- H^2 . The yield of 2-ethylnaphthalene-1- H^2 , prepared from 40 g. of 1-bromo-2-ethylnaphthalene as described for (d) above, is 13.5 g. (50%); b.p. 105° (6 mm.), n_D^{20} 1.60061, d_4^{20} 0.9967.

(g) 2-Methyl- H_1^2 -naphthalene. 2-(Bromomethyl)naphthalene, 22 g. (Note 4), is treated as in the preparation of 1-methylnaphthalene-4- H^2 . After recrystallization from methanol, the yield of 2-methyl- H_1^2 -naphthalene, m.p. 33.5° , is 4.5 g. (31%); b.p. 106° (7 mm.), n_D^{40} 1.60216, d_4^{40} 0.9974.

B. Notes

1. 1-Bromo-4-methylnaphthalene was prepared by the bromination of 1-methylnaphthalene in carbon disulfide.

2. 1-Ethylnaphthalene, b.p. 105° (5 mm.), was prepared¹ from 1-bromonaphthalene and brominated² in carbon disulfide to obtain 1-bromo-4-ethylnaphthalene, b.p. 123° (3 mm.), in 60% yield.

3. 1-Bromo-2-methylnaphthalene was prepared according to Mayer and Sieglitz.²

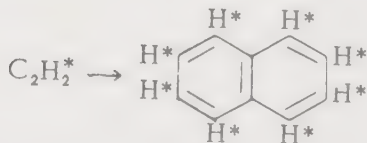
4. 2-Methylnaphthalene was brominated at 240° with a mercury lamp according to the procedure of Wislicenus and Elvert.³

¹H. Luther and G. Wächter, *Ber.*, 82, 161 (1949).

²F. Mayer and H. Sieglitz, *ibid.*, 55, 1835 (1922).

³W. Wislicenus and H. Elvert, *ibid.*, 49, 2820 (1916).

NAPHTHALENE- H_8^2



J. Goubeau, H. Luther, K. Feldmann and G. Brandes, *Ber.*, 86, 214 (1953).

A. Procedure (Note 1)

The acetylene- H_2^2 from the reaction of calcium carbide and 50 g. of water- H_2^2 is passed through the reaction tube (see Figure XVI, 6) at a rate of 4 l. per hour (Note 2). The temperature within the tube is maintained at approximately 1025° (Note 3). After the polymeric reaction products

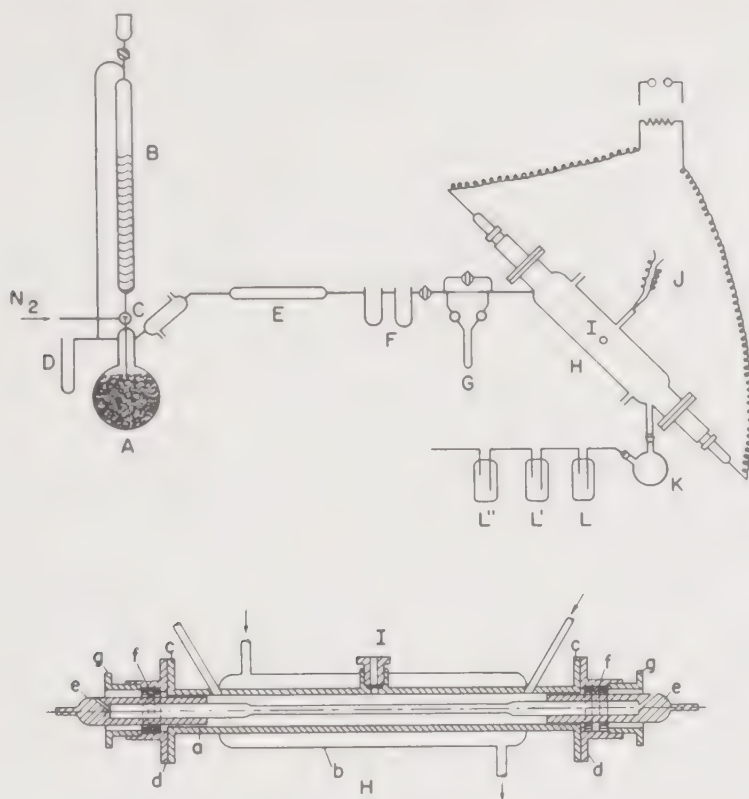


Fig. XVI, 6 Apparatus for polymerization of acetylene- H_2^2 to naphthalene- H_8^2 (J. Goubeau, H. Luther, K. Feldmann and G. Brandes). A, flask containing calcium carbide; B, compensated buret; C, 3-way stopcock; D, manometer; E, tube containing oxides of copper, manganese and silver, F, drying tubes; G, flow meter; H, polymerization tube; I, quartz window; J, thermocouple leads; K, product receiver; L-L'', cold traps; a, copper tube (430 mm. \times 50 mm.); b, copper cooling mantle; c, flange; d, outer flange; e, support for the silicon carbide rod; f, asbestos packing; g, packing nut.

are condensed in traps cooled with Dry Ice and acetone, the unreacted acetylene- H_2^2 is collected in a trap cooled with liquid nitrogen. This trap is then attached at the nitrogen inlet on the acetylene- H_2^2 generator, and the unreacted acetylene is recycled through the reaction tube. The acetylene- H_2^2 from an additional 50 g. of water- H_2^2 and fresh carbide is passed through the tube and recycled as before. The yield of condensate is 34 g. (65%, based on 75% of the water introduced). Fractionation of the condensate gives 18.2% of a light fraction (b.p. up to 170°), 26.9% of a middle fraction (b.p. $170-230^\circ$), 13.3% of a heavy fraction (b.p. $230-270^\circ$), and 41.4% of residue. The major part of the naphthalene- H_8^2 distills between 222° and 225° ; an additional amount is obtained by cooling the other fractions for a long period of time. The total yield of crude product is 6.5 g. (19.1%) (Note 4). After the naphthalene-

H_8^2 is recrystallized from carbon tetrachloride and sublimed *in vacuo*, it has the following properties: m.p. 80.2° (cor.); b.p. 217.1° (760 mm., cor.); d_4^{20} 1.242; n_D^{20} 1.58075.

B. Notes

1. The synthesis of aromatic hydrocarbons from acetylene at high temperature is according to the work of Berthelot¹ and has been investigated by others.²

2. The acetylene- H_8^2 was purified and freed of hydrogen sulfide by being passed through a tube containing a mixture of 46% copper oxide, 46% manganese dioxide and 8% silver oxide. The unpurified gas contained 0.9%, the purified gas 0.002% by volume of hydrogen sulfide. The purified gas was then dried by being passed through calcium chloride and phosphorus pentoxide tubes.

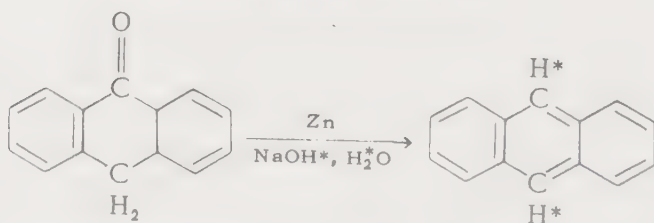
3. Preliminary experiments had shown this to be a favorable temperature for the formation of naphthalene. Based on a comparison of the dependence of the equilibrium constants on temperature for the two reactions, thermodynamically the pyrogenic formation of naphthalene from acetylene should be favored over the formation of benzene.

4. By fractional crystallization of the light fraction there was obtained 0.5 g. of yellow-colored benzene- H_8^2 , which was purified by treatment with sodium and vacuum distillation.

¹M. Berthelot, C. R. hebdom. Séances Acad., 111, 471 (1890).

²R. Schwarz, D. Pflugmacher, J. prakt. Chem., 156, 205 (1940); R. Schwarz, D. Pflugmacher and M. Ruhnke, *ibid.*, 161, 137 (1942); R. Schwarz, Ber., 75, 2012 (1942).

ANTHRACENE-9,10- H_8^2



V. Gold and F. A. Long, J. Am. Chem. Soc., 75, 4543 (1953).

A. Procedure

Anthrone is reduced to anthracene-9,10- H_8^2 essentially according to the procedure of Martin,¹ as in the following example.

Zinc dust, 25 g., is allowed to stand for a few minutes with an aqueous solution of 0.1 g. of copper sulfate crystals; the solution is poured

off, and to the activated metal is added 400 ml. of 2 *N* sodium hydroxide (Note 1), 100 ml. of toluene and 10 g. of anthrone. The mixture is heated with an oil-bath and gently refluxed for 12 hours (Note 2). The mixture is allowed to cool slightly, 100 ml. of benzene is added, and the liquid mixture is transferred to a separatory funnel, using 100 ml. more benzene to wash the residue of zinc. The hydrocarbon solution is treated with carbon while still wet, concentrated to a volume of 50–60 ml. and allowed to cool. The anthracene separates in thin, colorless plates, m.p. 216–216.5°; yield, 8.6 g. (93%) (Note 3).

B. Notes

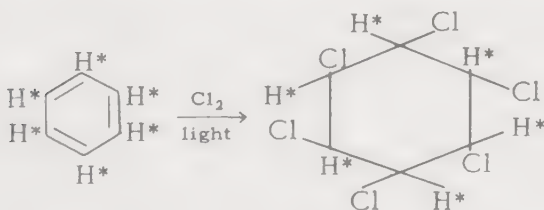
1. A mixture of water- H_2^2 and water (1:3) was used as the reaction medium instead of water.

2. The aqueous layer, which at first assumed a bright red color, turned yellow after about 3 hours, and finally both layers became colorless.

3. According to mass spectrometric analysis, the product of Gold and Long was a mixture of anthracene-9- H^2 and -9,10- H_2^2 , with 80% of the total deuterium in the sample (2.89%) present as anthracene-9- H^2 .

¹E. L. Martin, J. Am. Chem. Soc., 58, 1438 (1936).

1,2,3,4,5,6-HEXACHLOROCYCLOHEXANE- H^2



N. R. Trenner, R. W. Walker, B. Arison and R. P. Buhs, Anal. Chem., 21, 285 (1949).

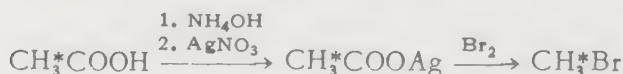
Procedure

Benzene- H_6^2 , 139 g., is treated with 150 g. of chlorine, under an atmosphere of nitrogen, and irradiated with a sun lamp. From the crude product, 184 g., 12 g. of pure γ -1,2,3,4,5,6-hexachlorocyclohexane- H_6^2 , m.p. 112.5°, obtained by fractional crystallization.¹ The crude material is treated with a limited amount of methanol, in which the α - and β -isomers are relatively insoluble. Separation of the solid product, I, leaves a solution containing the γ - and δ -isomers. On partial evaporation of the solution, a small crop of practically pure γ -isomer, II, is obtained. As evaporation is continued, the next crop of crystals contains γ -isomer and some

β -isomer. A third crop, III, contains γ -, a trace of β - and some δ -isomer. Pure γ -isomer is prepared from II by recrystallization from chloroform. Pure δ -isomer may be prepared with more difficulty from III by selective precipitation of a methanol solution with petroleum ether, followed by recrystallization from chloroform. Pure α - and β -isomers are readily obtained from I by taking advantage of the low solubility of β -isomer in practically all solvents.

¹R. E. Slade, Chem. & Ind. (London), 23, 314 (1945).

BROMOMETHANE- H_3^2



B. Nolin and L. C. Leitch, Can. J. Chem., 31, 153 (1953).

A. Procedure

(a) *Silver Acetate- H_3^2* . Acetic- H_3^2 acid is diluted with water, the solution is neutralized carefully with cold dilute ammonia, and silver nitrate solution is added. The precipitate of silver acetate- H_3^2 is collected, washed with cold water and dried (Note 1). The yield of the silver salt is practically quantitative (Note 2).

(b) *Bromomethane- H_3^2* . Silver acetate- H_3^2 (8.5 g., 0.05 mole) is placed in a 25-ml. flask, A, which is sealed to an S-shaped tube at B, Figure XVI, 7, and dried under vacuum in a boiling water-bath. Bromine (3.1 ml., 0.06 mole) is dried over phosphorus pentoxide and distilled under vacuum into the U-tube, C, cooled with liquid nitrogen. The evacuated reactor is sealed off above the U-tube at E. The bromine is melted by submerging the U-tube in an acetone-bath at room temperature, and the reaction flask is cooled with liquid nitrogen until the bromine begins to boil and some is frozen in the neck of the flask above the silver acetate. This portion of the bromine is melted and reacted with the silver acetate while the flask is swirled. This process, using small amounts of bromine, is repeated ten times (Note 3). Then the reaction flask is cooled, and some bromine is run down the neck of the flask from the U-tube and is frozen just above the bulb. The more volatile methyl- H_3^2 bromide is frozen in the U-tube, and the bromine is then allowed to melt and react with the silver acetate. The process is repeated until all the bromine is used up (Note 4). The reaction mixture is kept 10-15 hours in the dark.

The reaction flask is cooled with liquid nitrogen; the tube is opened and attached to the vacuum manifold by means of joint F. The system is completely evacuated, and all the volatile material is distilled into a clean receiver cooled with liquid nitrogen. The yellow residue is then

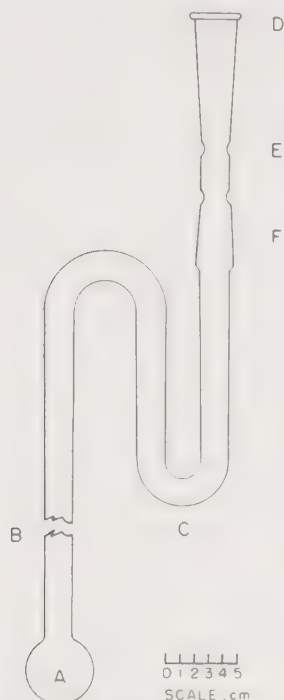


Fig. XVI, 7 Apparatus for the preparation of bromomethane- H_3^2 (B. Nolin and L. C. Leitch). A, flask containing silver acetate- H_3^2 ; B, point of sealing flask A to the S-tube; C, U-tube for bromine; D, standard taper joint for attachment to a vacuum manifold; E, restriction for sealing; F, standard taper joint for attachment to vacuum manifold.

heated to liberate any remaining methyl- H_3^2 bromide from the intermediate complex. The crude product is vacuum-distilled through a U-tube filled with Ascarite (Note 5). Passage through a second tube filled with Ascarite and Drierite removes the last traces of bromine and any water (Note 6). To remove by-products (Note 7), the material is cooled to -78° and its vapor is allowed to expand four times into the evacuated manifold; these fractions are discarded. Four distillations from a bath at -78° are then carried out from bulb to bulb to remove the less volatile impurities. The yield is 4.0 g., 81.7% (Note 8).

B. Notes

1. The silver acetate- H_3^2 in the filtrate is easily recovered by concentration.

2. If the silver salt is not free of acetic acid, it is purified by further washing or crystallization from water, since traces of the acid reduce the yield in the subsequent preparation of methyl- H_3^2 bromide.

3. By this time some methyl bromide has been formed.

4. The reaction time varies from 30 to 45 minutes and should not be lessened even when the reaction appears to run smoothly. It is prefer-

able to warm the reaction flask to room temperature before further addition of bromine; this avoids the accumulation of much undecomposed complex in the reaction mixture.

5. Carbon dioxide and most of the excess bromine are removed.

6. In some runs a yellow color persisted but was removed upon further treatment with Ascarite.

7. Vapor pressure measurements and mass spectrometric analyses indicated slight contamination with ethane, acetic acid and possibly methyl acetate.

8. The purification treatment yields a product of high chemical purity, as judged by its mass spectrum and vapor pressure at 0° (681.5 mm.). This value is in agreement with that reported by Beersmans and Jungers¹ for pure methyl bromide of comparable deuterium content. Mass analyses indicated an isotopic purity of 99.3 atom per cent deuterium (98.0% CH_3^2Br and 2.0% CHH^2Br).

C. Other Preparations

Bromomethane- H_3^2 has been prepared by heating *N*-methyl- H_3^2 -benzamide with phosphorus pentabromide² and by the reaction of methanol- H_4^2 and hydrogen bromide.¹ The first of these methods gave a low over-all yield, starting with nitromethane. The second required an elaborate vacuum apparatus, which is shown diagrammatically by Beersmans and Jungers;¹ the initial product, which contained 51% of a mixture of bromomethane- H_1^2 and bromomethane- H_2^2 and 49% of bromoethane- H_3^2 , was fractionated in a low-temperature column.

Bromomethane- H_1^2 has been prepared³ in about 93% isotopic purity from the direct action of hydrogen- H^2 bromide and diazomethane in butyl ether. It has also been prepared,⁴ in high yield and about 95% purity, by a procedure similar to that described for bromomethane- H_3^2 . Ketene was prepared⁵ and bubbled through water- H_2^2 . The resulting bis(acetic- H_1^2) anhydride was reacted with dry silver oxide to obtain silver acetate- H_1^2 , which was treated with bromine in carbon tetrachloride.

Langseth and Bak⁶ attempted to prepare bromomethane- H_1^2 , in a similar manner, from acetic- H_1^2 acid *via* silver acetate- H_1^2 , but the product was a mixture of bromomethane, bromomethane- H_1^2 , bromomethane- H_2^2 and bromomethane- H_3^2 .

¹J. Beersmans and J. C. Jungers, *Bull. soc. chim. Belges*, 56, 238 (1947).

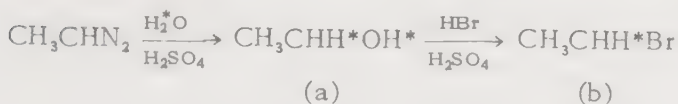
²H. D. Noether, *J. Chem. Phys.*, 10, 664 (1942).

³F. I. Andersen, *Proc. Conf. Applications Isotopes Sci. Research*, Univ. Melbourne, 1950, pp. 195-204; *Chem. Abstracts*, 45, 9389 (1951).

⁴F. I. Andersen, *Nature*, 173, 541 (1954).

⁵J. W. Williams and C. D. Hurd, *J. Org. Chem.*, 5, 122 (1940).

⁶A. Langseth and B. Bak, *Kgl. Danske Videnskab. Selskab, Mat.-fvs. Medd.*, 24, No. 3 (1947).

1-BROMOETHANE-1-H₁²

A. Langseth and B. Bak, Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd., 24, No. 3 (1947).

A. Procedure

(a) *Ethanol-H²-1-H₁²*. A solution of 0.5 mole of diazoethane in dry ether (Note 1) is added dropwise to a mixture of 25 ml. of water-H₂² and 5 ml. of concentrated sulfuric acid (Note 2). When the evolution of nitrogen ceases, ether is removed, through a 100-cm. column, from the mixture of ethanol-H²-1-H₁², ethanol-1-H₁², ethanol-H², ethanol and sulfuric acid esters. The latter are saponified, and the alcohol mixture is removed by distillation.

(b) *1-Bromoethane-1-H₁²*. Treatment of the above mixture of alcohols with hydrobromic and sulfuric acids gives 18 g. of 1-bromoethane-1-H₁², b.p. 38.40–38.60° (760 mm.).

B. Notes

1. During drying of the diazoethane solution over solid potassium hydroxide some decomposition occurred, giving some ordinary ethanol.

2. The use of sulfuric acid rather than sulfuric acid-H₂² resulted in about 4% ordinary ethanol in the product.

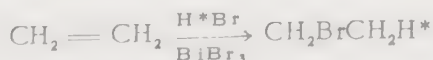
C. Other Preparations

Ethanol-H²-1-H₁² has been prepared by the reduction of acetaldehyde with hydrogen-H₂² and a nickel catalyst¹ and with a platinum catalyst.²

Ethanol-1-H₁² was obtained¹ by the treatment of ethanol-H²-1-H₁² with calcium hydroxide.

¹T. Yosida and T. Titani, Bull. Chem. Soc. Japan, 14, 125 (1941).

²L. C. Anderson and N. W. MacNaughton, J. Am. Chem. Soc., 64, 1456 (1942).

1-BROMOETHANE-2-H₁²

K. Graupner and E. R. S. Winter, J. Chem. Soc., 1952, 1145

Procedure

1-Bromoethane-2- H_1^2 is prepared by the addition of hydrogen- H^2 bromide to ethylene in the presence of a bismuth bromide-asbestos catalyst in accordance with the following procedure of Wibaut.¹ Ethylene is passed through concentrated sulfuric acid and then through a tube containing anhydrous calcium chloride where it is mixed with hydrogen bromide. The mixture of gases is passed over the catalyst of bismuth tribromide on purified asbestos (Note 1) at a temperature of 20° . The mixture of gases issuing from the reaction tube is passed through a wash-bottle filled with water (Note 2) and then through a series of U-tubes filled with soda lime. The product is condensed in cold traps at -78° and, in order to collect all the alkyl halide, air is passed through the apparatus for 2 hours at the end of each preparation (Note 3). The yield of ethyl bromide in a 2.5-hour period, using 0.5 liter of ethylene per hour, is 6.6 g. (84%) (Note 4).

B. Notes

1. Bismuth trioxide is dissolved in concentrated hydrobromic acid, and pure asbestos is added to the solution. This mixture is evaporated until nearly dry, filled into the 100×23 -cm. reaction tube and heated at 180 – 200° for 8 hours in a current of dry hydrogen bromide.

2. Most of the excess hydrogen bromide is removed in the water-wash.

3. In the preparation of ethyl bromide it was necessary to warm the U-tubes to prevent loss of some of the product.

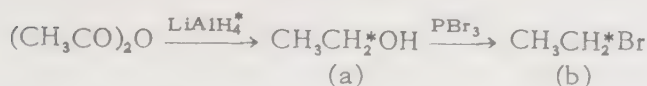
4. Graupner and Winter report only a 26% yield of 1-bromoethane-2- H_1^2 by this procedure. The product, b.p. 38 – 40° , was purified by distillation from phosphorus pentoxide.

C. Other Preparations

Langseth and Bak² have prepared 1-bromoethane-2- H_1^2 by the reaction of ethylene and hydrogen- H^2 bromide at 200° over a bismuth bromide catalyst. The yield of product, b.p. 38.30 – 38.70° (760 mm.), was 15%. They have also prepared 1-bromoethane-2- H_1^2 , in 90% yield, by heating 1-iodoethane-2- H_1^2 with cupric bromide for 4 hours under reflux.

¹J. P. Wibaut, J. J. Diekmann and A. J. Rutgers, *Rec. trav. chim.*, **47**, 477 (1928).

²A. Langseth and B. Bak, *Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd.*, **24**, No. 3 (1947).

1-BROMOETHANE-1-H₂²

V. J. Shiner, Jr., J. Am. Chem. Soc., 75, 2925 (1953).

A. Procedure

(a) *Ethanol-1-H₂²*. A solution of 4.3 g. (0.043 mole) of acetic anhydride in 25 ml. of diethyl carbitol is added slowly to an ice-cold slurry of 0.2 g. of lithium aluminum hydride-H₄² in 100 ml. of diethyl carbitol. The reaction mixture is kept overnight and then heated at 100° for several hours. After it is cooled, the reaction mixture is treated with 50 ml. of 2-(2-butoxyethoxy)ethanol, and the resulting ethanol-1-H₂² is removed by fractional distillation. The yield is 3.5 g. (89%).

(b) *1-Bromoethane-1-H₂²*. To 3.5 g. of ethanol-1-H₂², cooled to -10°, is added dropwise 8 g. (0.03 mole) of phosphorus tribromide. The reaction mixture is fractionally distilled, and the product is dried over potassium carbonate; yield, 7.0 g. (84%).

B. Other Preparations

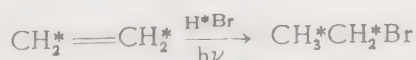
Ethanol-1-H₂² has been prepared by the treatment of ethyl-1-H₂² bromide with water or silver sulfate¹ and by lithium aluminum hydride-H₄² reduction of phenyl acetate.²

1-Bromoethane-1-H₂² has been prepared³ by the brominative degradation of silver propionate-2-H₂². 1-Bromoethane-2-H₃² has been prepared³ similarly from silver propionate-3-H₃².

¹J. W. Cornforth and G. Popják, *Nature*, 164, 1053 (1949).

²F. H. Westheimer, H. F. Fisher, E. E. Conn and B. Vennesland, J. Am. Chem. Soc., 73, 2403 (1951).

³B. Nolin, *Can. J. Chem.*, 31, 1257 (1953).

BROMOETHANE-H₃²

L. C. Leitch and A. T. Morse, *Can. J. Chem.*, 31, 785 (1953).

A. Procedure

An apparatus described earlier by Leitch and Morse¹ (see 1,2-dibromoethane-H₄², Note 1), in which the reactor is equipped with a quartz window, is evacuated, and hydrogen-H² bromide and ethylene-H₄² are intro-

duced in a ratio of 1:1. The mixture is irradiated with ultraviolet light from a Hanovia lamp. A bath kept at -40° is placed around the receiver at the bottom of the reactor to condense the bromoethane- H_5^2 as it is formed. The bromoethane- H_5^2 is washed with ice-cold water and distilled *in vacuo*, first through soda lime and then through phosphorus pentoxide. The yield of product (99.3 atom per cent H^2 , n_D^{20} 1.4211) is nearly quantitative. The vapor pressure of bromoethane- H_5^2 , measured between -20° and $+16^{\circ}$ in an apparatus similar to that used by Booth and Swinehart,² is given in Table XVI, 7.

TABLE XVI, 7
Vapor Pressures of Bromoethane and Bromoethane- H_5^2

$\text{C}_2\text{H}_5\text{Br}$		$\text{C}_2\text{H}_5^2\text{Br}$		$\text{C}_2\text{H}_5^3\text{Br}$	
Temp., $^{\circ}\text{C}$.	P, cm.*	Temp., $^{\circ}\text{C}$.	P, cm.	Temp., $^{\circ}\text{C}$.	P, cm.
-20.0	5.90	-20.0	6.70	-6.0	13.65
-10.0	10.10	-17.0	7.63	-1.0	17.14
0.0	16.50	-14.5	8.69	+1.5	19.72
+10.0	25.69	-12.0	10.14	+7.0	24.66
+20.0	38.59	-10.0	11.27	+12.0	30.83
		-7.5	12.53	+16.0	36.14

*The bromoethane values are from International Critical Tables.

B. Other Preparations

Bromoethane- H_5^2 has been prepared by the addition^{3,4} of hydrogen- H^2 bromide to ethylene- H_4^2 (the reaction was accelerated by ultraviolet light) and by the treatment⁵ of ethanol- H_6^2 with a mixture of hydrobromic and sulfuric acids.

¹L. C. Leitch and A. T. Morse, Can. J. Research, 30B, 924 (1952).

²H. S. Booth and C. F. Swinehart, J. Am. Chem. Soc., 57, 1333 (1935).

³M. de Hemptinne and C. Velghe, Physica, 5, 958 (1938).

⁴M. de Hemptinne, J. C. Jungers and J. M. Delfosse, J. Chem. Phys., 6, 319 (1938).

⁵A. Langseth and B. Bak, Kgl. Danske Videnskab. Selskab, Mat.-fys. Medd., 24, No. 3 (1947).

H^2 -BROMOETHANES AND H^2 -BROMOETHYLENES

J. C. Jungers and J. Verhulst, Acad. roy. Belg., Classe sci., Mem., 23, 3-44 (1949).

A. Procedure

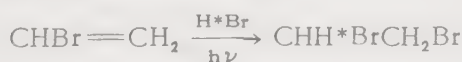
1. *H^2 -1,2-Dibromoethanes.* The photochemical addition of hydrogen bromide or hydrogen- H^2 bromide to acetylene or acetylene- H_2^2 , when ir-

radiated with a mercury arc (Note 1), results in the formation of 1,2-dibromoethanes in better than 90% yields. The apparatus (Note 2) consists of a Pyrex glass vacuum manifold to which are attached large storage flasks for hydrogen- H^2 bromide and acetylene and at least two reaction flasks, each equipped with a manometer. Hydrogen bromide and acetylene are transferred to the reaction flasks in a ratio of 2 moles to 1, respectively (Note 3). The reaction is inhibited by iodine and iodine-containing compounds, is catalyzed by traces of aldehydes, ketones and oxygen, but is not affected by carbon disulfide, 1,2-dibromoethane or 1,1,2-tribromoethane (Note 4).

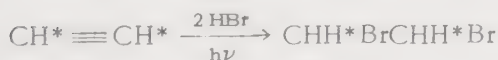
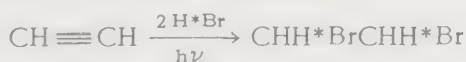
The addition of hydrogen bromide to vinyl bromide takes place much more readily than the corresponding reaction with acetylene. There is no induction period, and the reaction progresses very rapidly (approximately five times as fast as the corresponding reaction with acetylene under the best conditions). According to kinetic studies, at a total pressure of 75 cm., the maximum reaction rate is attained with a ratio of 40 cm. of vinyl bromide to 30 cm. of hydrogen bromide.

The H^2 -1,2-dibromoethanes prepared by the two above methods, according to the following reactions, are given with physical data in Table XVI, 8 (Note 5).

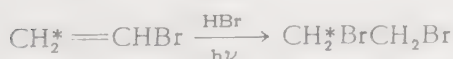
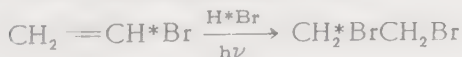
(a) 1,2-Dibromoethane- H_1^2 .



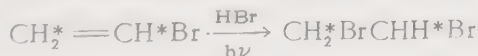
(b) 1,2-Dibromoethane-1,2- H_2^2 .



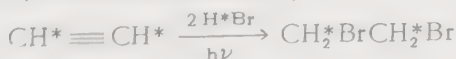
(c) 1,2-Dibromoethane-1- H_2^2 .



(d) 1,2-Dibromoethane- H_3^2 .



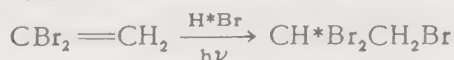
(e) 1,2-Dibromoethane- H_4^2 .



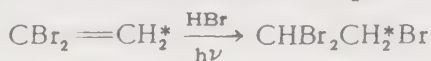
2. H^2 -1,1,2-Tribromoethanes. The tribromoethanes are prepared either by the photochemical addition of hydrogen bromide or hydrogen- H^2 bromide to the appropriate vinylidene bromide or bromoethylene, or by the bromi-

nation of suitable bromoethylenes. The 1,1,2-tribromoethanes prepared by the above methods, according to the following reactions, are given in Table XVI, 8 (Note 6).

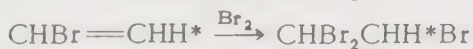
(f) 1,1,2-Tribromoethane-1- H^2 .



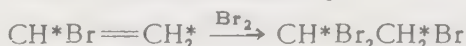
(g) 1,1,2-Tribromoethane-2- H^2 .



(h) 1,1,2-Tribromoethane-2- H_1^2 .



(i) 1,1,2-Tribromoethane- H_3^2 .



3. H^2 -Bromoethylenes. The monobromoethylenes containing deuterium are prepared either by the photochemical addition of hydrogen bromide to acetylene or by the removal of bromine from the appropriate tribromoethane with zinc.

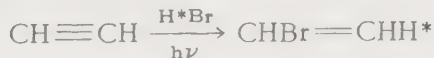
In the photochemical method, practically quantitative yields of the vinyl bromides are obtained by preventing the addition of a second molecule of hydrogen bromide to the product obtained from acetylene. The apparatus (Note 7) used in this synthesis is constructed in such a manner as to permit irradiation of the reaction mixture and the elimination of vinyl bromide as it is formed. Hydrobromic acid is first admitted to the apparatus and condensed in a trap cooled with a refrigerant mixture at -80° . Acetylene, which dissolves in great quantity in the cold liquid hydrogen bromide, is then introduced. The major part of the reaction mixture is thus held in reserve, in the liquid form, while a sufficient amount to sustain the reaction is circulated through the apparatus in the gaseous phase. A thermo-siphon, consisting of an electric furnace which encloses a vertical arm of the apparatus, keeps the gases in circulation through the reaction chamber, where they are subjected to irradiation. The reaction mixture then passes through the cold trap where vinyl bromide is condensed from the gaseous stream of reactants. In this manner the contents of the trap are continuously enriched in vinyl bromide as they become poor in reactants. Finally the product is subjected to distillation in a column of special design (Note 7). The column, which contains a metal spiral, is vacuum-jacketed. At its upper end the jacket is enlarged to contain a refrigerant at the desired temperature. During the distillation, the uncombined acetylene and hydrobromic acid first escape and are returned to the reaction apparatus. The vinyl bromide is then distilled, washed with water and stored either as a gas or a liquid (Note 8).

TABLE XVI, 8
H²-Bromoethanes

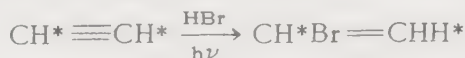
Formula	Compound	M.p., °C.	Boiling Points, °C.		Impurity, %	Density, d ₄ ¹⁵		n _D ³⁰		Mol. Ref. Cor.
			Observed/mm.	At 760 mm.		Observed	Cor.	Observed	Cor.	
CH ₂ BrCH ₂ Br	1,2-Dibromoethane	10.0-10.1	131.87/766.6	131.55	2.18907	1.53310	27.027
(a) CHH*BrCH ₂ Br	1,2-Dibromoethane-H ₂ ¹	10.1-10.2	131.55/766.5	131.25	H ₂ —H ₂ ,	2.20037	2.20079	1.53267	1.53264	27.003
(b) CHH*BrCHH*Br	1,2-Dibromoethane-1,2-H ₂ ²	10.2-10.3	131.30/766.4	130.90	H ₂ —H*H, 2	2.21310	2.21251	1.53223	1.53222	26.980
(c) CH ₂ *BrCH ₂ Br	1,2-Dibromoethane-1-H ₂ ³	10.2-10.3	131.25/766.7	130.90	H ₂ —H*H, 8	2.21197	2.21251	1.53230	1.53227	26.988
(d) CH ₂ *BrCHH*Br	1,2-Dibromoethane-H ₂ ³	10.3-10.4	130.47/759.6	130.50	$\left. \begin{array}{l} \text{HH*—HH*} \\ \text{H}_2\text{—H}_2^*, \end{array} \right\} 7$	2.2383	2.22423	1.53149	1.53114	26.953
(e) CH ₂ *BrCH ₂ *Br	1,2-Dibromoethane-H ₂ ⁴	10.4-10.5	$\left\{ \begin{array}{l} 130.15/760.0 \\ 130.45/766.2 \end{array} \right.$	130.15		2.23583	2.23595	1.53080	1.53077	26.925
CHBr ₂ CH ₂ Br	1,1,2-Tribromoethane	d ₄ ²⁰		n _D ²⁰		
(f) CH*Br ₂ CH ₂ Br	1,1,2-Tribromoethane-1-H ₂ ¹	H ₂ —H, 8	2.63314	2.62205	1.59325	34.491
(g) CHBr ₂ CH ₂ *Br	1,1,2-Tribromoethane-2-H ₂ ²	HH*—H, 6	2.64219	2.63170	1.59268	1.59263	34.465
(h) CHBr ₂ CHH*Br	1,1,2-Tribromoethane-2-H ₂ ¹	H ₂ —H, 3	2.65113	2.64061	1.59197	1.59191	34.443
(i) CH*Br ₂ CH ₂ *Br	1,1,2-Tribromoethane-H ₂ ³	H*H—H*, 1	2.64428	2.63340	1.59314	1.59313	34.466
			2.66197	2.65088	1.59147	1.59147	34.418

The H^2 -bromoethylenes are prepared by the above methods, according to the following reactions:

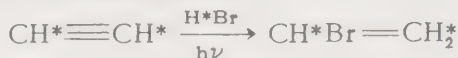
(k) *1-Bromoethylene-2- H_1^2* .



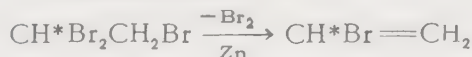
(l) *1-Bromoethylene-1,2- H_2^2* .



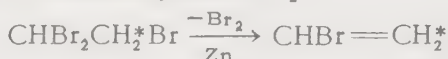
(m) *1-Bromoethylene- H_3^2* .



(n) *1-Bromoethylene-1- H^2* .



(o) *1-Bromoethylene-2- H_2^2* .



4. *H^2 -Vinylidene Bromides.* These compounds are formed from H^2 -tribromoethanes by removal of a molecule of hydrogen bromide under the influence of an equimolar mixture of potassium acetate and potassium carbonate in alcohol, according to the following procedure of Verhulst, Hemelrijck and Jungers.¹

(p) *1,1-Dibromoethylene- H_1^2* .



(q) *1,1-Dibromoethylene- H_2^2* .



Under the best conditions, a mixture of 67 g. of the tribromoethane, 24.5 g. of potassium acetate, 35 g. of potassium carbonate and 200 ml. of 95% alcohol is heated for 4 hours. During this time, the product is continuously distilled off with alcohol as an azeotrope. A first fraction distills up to a temperature of 72° , and a second fraction is obtained over a range of 72° to 78° . Redistillation of the two fractions yields 29 g. and 12.5 g., respectively, making the total yield 89–90%.

B. Notes

1. In preliminary experiments, it appeared that the reaction was influenced by light of longer wave length than that transmitted by Pyrex glass, i.e., 3000 Å. Since hydrogen bromide and acetylene both absorb light of less than 2500 Å, it was indicated that the reaction was catalyzed by the action of light on traces of impurities.

2. A diagram of the apparatus is given by Jungers and Verhulst.
3. Kinetic studies indicated that the maximum reaction rate was attained with the stoichiometric ratio of reactants. An excess of hydrogen bromide has less unfavorable effect on the reaction rate than an excess of acetylene.
4. The reaction with acetylene has an induction period which is apparently a function of the impurities in any given batch of the two reactants.
5. The physical constants for these compounds were reported by Verhulst and Jungers.²
6. The physical constants for these compounds were also reported by Verhulst and Jungers.² Molar heat capacities of H^2 -1,2-dibromoethanes and H^2 -1,1,2-tribromoethanes have been reported by Wuyts-Robiette³ and by d'Hont.⁴
7. A diagram of this apparatus, including a special distillation column, is also given by Verhulst and Jungers.²
8. The storage of vinyl bromide presents difficulties, since this compound shows a marked tendency to polymerize. Pyrogallol was found to be an excellent stabilizer, as samples of vinyl bromide were kept in a monomeric form for 2 years. During this time, some of the samples were subjected to irradiation with light of wave lengths up to 4000 Å. Non stabilized samples showed turbidity at the end of an hour under similar conditions.

C. Other Preparations

1,2-Dibromoethane-1,2- H_2^2 (b.p. 130–135°) has been prepared by Patterson and du Vigneaud⁵ in 63% yield (based on water- H_2^2) by the bromination of ethylene-1,2- H_2^2 , which was obtained by the reduction of acetylene- H_2^2 with chromous chloride.

From *trans*-ethylene-1,2- H_2^2 , two samples of 1,2-dibromoethane-1,2- H_2^2 have been prepared.⁶ The reaction of *trans*-ethylene-1,2- H_2^2 with a degassed aqueous solution saturated with bromine and potassium bromide, at 0° in the absence of light, gave 95% *meso*- and 5% *racemic*-1,2-dibromoethane-1,2- H_2^2 . Bromination in carbon tetrachloride at room temperature in the presence of light gave a mixture of 50% *meso*- and 50% *racemic*-1,2-dibromoethane-1,2- H_2^2 .

¹J. Verhulst, F. Van Hemelrijck and J. C. Jungers, *Natuurw. Tijdschrift. (Belg.)*, 25, 203 (1943).

²J. Verhulst and J. C. Jungers, *Bull. soc. chim. Belges*, 58, 73 (1949).

³J. Wuyts-Robiette and J. C. Jungers, *ibid.*, 58, 80 (1949).

⁴M. d'Hont and J. C. Jungers, *ibid.*, 58, 196 (1949).

⁵W. I. Patterson and V. du Vigneaud, *J. Biol. Chem.*, 123, 327 (1938).

⁶H. J. Bernstein, A. D. E. Pullin, B. S. Rabinovitch and N. R. Larson, *J. Chem. Phys.*, 20, 1227 (1952).

1,2-DIBROMOETHANE- H_4^2 

L. C. Leitch and A. T. Morse, Can. J. Research, 30B, 924 (1952).

A. Procedure

At the start of the experiment, the apparatus (Note 1) is evacuated to 1 mm. or less and flushed with the first of the hydrogen- H_2^2 bromide generated from water- H_2^2 and phosphorus tribromide. Colorless hydrogen- H_2^2 bromide, 10-20 ml., is generated, passed through a trap at -40° and collected in a storage flask cooled to -78° . Hydrogen- H_2^2 bromide is then distilled into the reactor (2-liter) until the pressure is 500 mm. Acetylene- H_2^2 is added, until the pressure is nearly atmospheric, followed by a few mm. of dry air. After a variable induction period or irradiation for a few minutes with ultraviolet light (Note 2), the manometer begins to rise, and the surface of the reactor becomes coated with condensed dibromoethane. The ratio of hydrogen- H_2^2 bromide to acetylene is kept at 2:1 during the entire reaction. The crude product is dissolved in methylene chloride, shaken with dilute potassium carbonate solution until free of acid, dried over solid potassium carbonate and distilled. The yield of 1,2-dibromoethane- H_4^2 , b.p. 129.5° , is nearly quantitative; d_4^{20} 2.2266, n_D^{20} 1.5360 (Note 3).

B. Notes

1. The all-glass apparatus, shown diagrammatically by Leitch and Morse, was comprised of a hydrogen- H_2^2 bromide generator with cold trap and storage flask, a reactor similar to that described by Taylor and Morey,¹ and an acetylene reservoir (see Figure XVI, 8).

2. Wilson and Wylie² reported the preparation of 1,2-dibromoethane- H_4^2 , of high isotopic purity, from acetylene- H_2^2 and hydrogen- H_2^2 bromide in the presence of activated carbon at 180° . In their procedure a large volume of hydrogen- H_2^2 bromide was required to remove adsorbed hydrogen from the catalyst and to activate the latter; their best yield was about 70%. Jungers and Verhulst³ prepared large amounts of 1,2-dibromoethane- H_4^2 , in quantitative yield, by the photochemical addition of hydrogen- H_2^2 bromide to acetylene. Since neither reactant absorbs light of the wavelength used, they assumed that traces of impurities in the acetylene had effected the reaction. In the absence of sunlight no reaction between acetylene and fuming hydrobromic acid was observed at 100° over a period of several months. Wibaut⁴ likewise observed no reaction at 100° , but Bauer⁵ patented the preparation of 1,2-dibromoethane, in excellent yields, from acetylene and hydrogen bromide either under the influence of

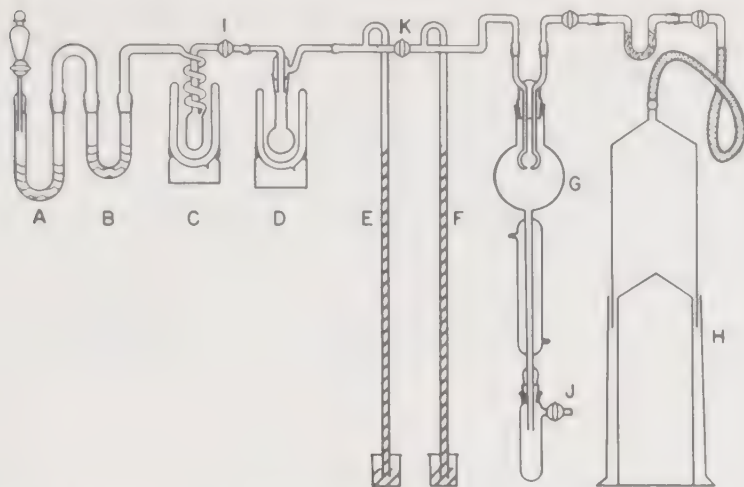


Fig. XVI, 8 Apparatus for preparation of 1,2-dibromoethane- H_4^2 (L. C. Leitch and A. T. Morse). A and B, U-tubes containing phosphorus tribromide; C, trap at -40° ; D, trap at -78° ; E and F, manometers; G, reactor; H, gas holder containing acetylene- H_2^2 over mercury; J, stopcock connected to vacuum pump.

sunlight or in the presence of oxygen or ozone. It was found by Leitch and Morse that to obtain rapid reaction using acetylene generated from calcium carbide and water it was necessary to purify the acetylene by bubbling it through concentrated sulfuric acid. When this treatment was omitted, reaction was very sluggish and eventually stopped. Fortunately no loss of hydrogen- H^2 by exchange was detected in the acetylene- H_2^2 after treatment with sulfuric acid.

3. The product analyzed 98.0 mole per cent $\text{C}_2\text{H}_4^2\text{Br}_2$ and 2.0% $\text{C}_2\text{H}_3^2\text{HBr}_2$ by mass spectrometry.

C. Other Preparations

1,2-Dibromoethane- H_4^2 has been prepared from acetylene- H_2^2 and hydrogen- H^2 bromide: in the presence of activated carbon;¹ under ultraviolet illumination;^{2,6-10} and over a catalyst of mercuric bromide on asbestos at 120° .¹¹

¹R. F. Taylor and G. H. Morey, *Ind. Eng. Chem.*, **40**, 432 (1948).

²C. L. Wilson and A. W. Wylie, *J. Chem. Soc.*, 1941, 596.

³J. C. Jungers and J. Verhulst, *Acad. roy. Belg., Classe sci., Mem.* **23** (2), 1 (1949).

⁴J. Wibaut, *Rec. trav. chim.*, **50**, 313 (1931).

⁵W. Bauer, German patent 368,467; 394,194 (1925).

⁶J. Verhulst, J. C. Jungers and M. de Hemptinne, *Natuurw. Tijdschr.*, **22**, 56 (1940); through *Chem. Abstracts*, **37**, 2335 (1943).

⁷J. C. Jungers and J. Verhulst, *Acad. roy. Belg., Classe sci. Mem.*, **23**, No. 2 (1949); through *Chem. Abstracts*, **45**, 4638 (1951).

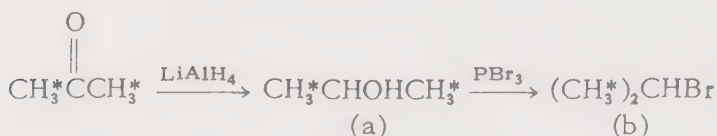
⁸J. Verhulst and J. C. Jungers, *Bull. soc. chim. Belges*, **58**, 73 (1949).

⁹J. T. Neu and W. D. Gwinn, J. Chem. Phys., 18, 1642 (1950).

¹⁰M. de Hemptinne, J. C. Jungers and J. M. Delfosse, J. Chem. Phys., 6, 319 (1938).

¹¹S. Mizushima and Y. Morino, Proc. Indian Acad. Sci., 8A, 315 (1938).

2-BROMOPROPANE-1,3-H₆²



V. J. Shiner, Jr., J. Am. Chem. Soc., 74, 5285 (1952).

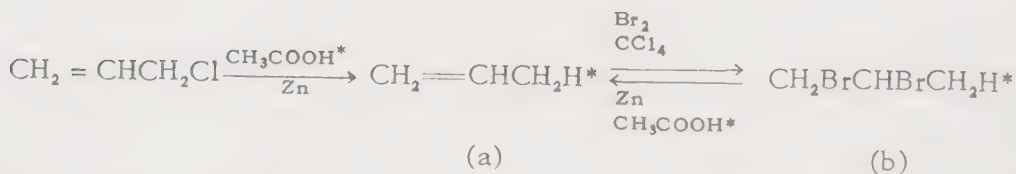
Procedure

(a) *2-Propanol-1,3-H₆²*. 2-Propanone-H₆², 4.0 g. (0.069 mole), is reduced with a slurry of 1.0 g. (0.025 mole) of lithium aluminum hydride¹ in 50 ml. of diethyl carbitol. The yield of 2-propanol-H₆² which is distilled from the reaction mixture is 3.0 g. (0.05 mole).

(b) *2-Bromopropane-1,3-H₆²*. To the above alcohol, cooled to -10°, is added dropwise 8 g. (0.03 mole) of phosphorus tribromide. After the reaction mixture is kept overnight, 3.6 g. (0.03 mole) of the isopropyl bromide is recovered by distillation, washed twice with cold sulfuric acid, dried with potassium carbonate and redistilled.

¹R. F. Nystrom, W. H. Yanks and W. G. Brown, J. Am. Chem. Soc., 70, 441 (1948).

1,2-DIBROMOPROPANE-3-H₁²



C. D. Hurd and J. L. Azorlosa, J. Am. Chem. Soc., 73, 33 (1951).

A. Procedure

(a) *Propene-3-H₁²*. Acetic acid-H₂², from 10.06 g. of water-H₂² (99.5%) and 56.6 g. of acetic anhydride, 400 ml. of dioxane (dried over sodium) and 140 g. of zinc dust are placed in a 3-necked flask equipped with dropping funnel, condenser and mercury-sealed stirrer. During two hours, 115 g. of allyl chloride is added. The evolved gas is liquified at -78° and distilled through a Davis column. The propene-3-H₁² fraction, 19 l., boiling

at -47° to -46° (atmospheric pressure), is passed into a large gas reservoir over sodium chloride solution.

(b) *1,2-Dibromopropane-3-H₁²*. The 19 l. of propene-3-H₁² is passed, during six hours, into an excess of bromine in carbon tetrachloride solution at -5° . The excess bromine is removed by shaking the cold solution with crushed ice and sodium bicarbonate. The organic layer is separated, washed three times with sodium bicarbonate solution, then with water, and dried over calcium chloride. Distillation of the solution yields 169.5 g. of 1,2-dibromopropane-3-H₁², b.p. $66-67^{\circ}$ (60 mm.). The yield is 75% based on water-H₂² used to prepare acetic acid-H².

B. Other Preparations

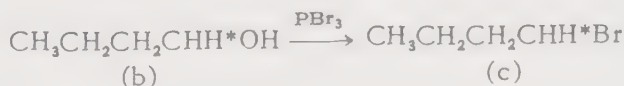
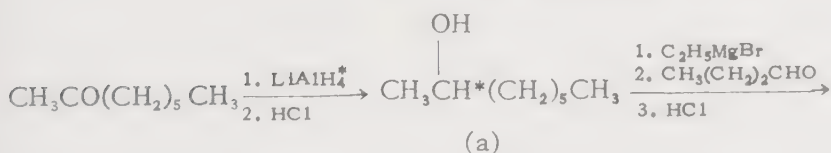
Propene-3-H₁² has been prepared in 78% yield by the action of zinc dust on 1,2-dibromopropane-3-H₁² in refluxing ethanol.¹

Propene-1-H₁² has been obtained in low yield, by the reduction of propyne-1-H² with copper-activated zinc and 1 N hydrochloric acid.²

¹C. D. Hurd and J. L. Azorlosa, J. Am. Chem. Soc., 73, 33 (1951).

²B. S. Rabinovitch and F. S. Looney, *ibid.*, 75, 2652 (1953).

1-BROMOBUTANE-1-H₁²



A. Streitwieser, Jr., J. Am. Chem. Soc., 75, 5014 (1953).

A. Procedure (Note 1)

(a) *2-Octanol-2-H²*. To 5.0 g. of lithium aluminum hydride-H₄² (Note 2) in 500 ml. of dry ether is added 64 g. of freshly distilled 2-octanone. After completion of the addition, the viscous solution is refluxed, with stirring, for several hours. To the cooled solution, concentrated hydrochloric acid is added until the inorganic salts precipitate. The ether solution is separated and washed successively with dilute hydrochloric acid, water and 10% sodium carbonate; then it is dried over anhydrous potassium carbonate. After removal of ether on a steam-bath, the residue is distilled (Note 3), and 59.6 g. (91.1%), b.p. $175-178^{\circ}$, is collected; d_4^{25} 0.8212. The DL-2-octanol-2-H² is resolved *via* the brucine salts of the hydrogen phthalate esters.¹

(b) *1-Butanol-1-H₁²* (Note 4). To the Grignard reagent prepared from 13.7 g. of ethyl bromide and 3.0 g. of magnesium in 100 ml. of ether (still containing traces of water) is added 16.5 g. (0.126 mole) of 2-octanol-2-H²; $\alpha_D -3.69^\circ$ ($l = 0.5$). The resulting solution is filtered through a sintered glass funnel into a glass-stoppered graduate. After the addition of 9.1 g. (0.126 mole) of freshly distilled butyraldehyde and dry ether to a total volume of 190 ml., the graduate is stoppered and shaken, with cooling. After 3 days at room temperature, the mixture is decomposed by the addition of dilute hydrochloric acid. The ether layer, washed successively with dilute hydrochloric acid, water and saturated sodium carbonate, is dried over anhydrous potassium carbonate. After removal of ether on a steam-bath, the residue is distilled through a Vigreux column, and 6.4 ml. of product, b.p. $95-123^\circ$, is collected (Note 5). The distillate, dried with potassium carbonate, is redistilled, and two fractions are collected: 1) b.p. $102-116^\circ$, 1.3 ml., $\alpha_D + 0.034 \pm 0.007^\circ$ ($l = 2$); 2) b.p. $116-117^\circ$, 2.3 ml., $\alpha_D + 0.079 \pm 0.007^\circ$ ($l = 4$), d_4^{25} 0.8166. The yield of *1-butanol-1-H₁²* is 29%.

(c) *1-Bromobutane-1-H₁²*. A portion of the *1-butanol-1-H₁²* from the above preparation (1.63 g., 0.021 mole) is cooled with Dry Ice, and 2.0 g. (0.0074 mole) of phosphorus tribromide is added. The flask, equipped with a reflux condenser and drying tube, is warmed slowly in a water-bath. After the initial exothermic reaction accompanying mixing of the two layers, the mixture is refluxed for 1 minute, by which time the copious evolution of hydrogen bromide has nearly subsided. Cold water is added, the mixture is centrifuged, the water layer is removed with a pipet and the butyl bromide layer is washed with cold water and with cold 10% sodium carbonate. After it is dried with sodium sulfate, the *1-bromobutane-1-H₁²* is distilled; the yield is 1.44 g. (48%), b.p. $96-103^\circ$, n_D^{20} 1.4340 and $\alpha_D 0.138 \pm 0.007^\circ$ ($l = 2$).

B. Notes

1. Substitution of a deuterium atom for hydrogen, in an ordinary primary compound, renders a secondary carbon atom asymmetric and hence potentially optically active. That such hydrogen-deuterium asymmetry can effect appreciable optical activity has been demonstrated in theory by Fickett² and experimentally in the systems ethyl-1-H₁²-benzene,³ menthane-3-H₁²,⁴ and menthane-2,3-H₂².⁵ Of the various possible approaches to the preparation of optically active *1-butanol-1-H₁²*, introduction of a deuterium atom into butyraldehyde by a stereospecific reduction was the method selected. The Meerwein-Ponndorf reduction has been shown to be partially stereospecific by Doering and Young.⁶ A very similar oxidation-reduction reaction between alkoxymagnesium halides and ketones⁶ should also be partially stereospecific and was the method employed.

2. Available from Metal Hydrides Inc., Beverly, Mass.

3. A silvered, vacuum-jacketed Vigreux column fitted with a partial take-off head was used.

4. Both the D- and L-forms of 2-octanol-2-H² were used in this work, giving rise, in each instance, to the 1-butanol-1-H₁² of opposite rotation.

5. The foreruns always contained water, probably from the dehydration of condensation products.

C. Other Preparations

Streitwieser has also prepared 1-butanol-1-H₁² in 32% yield⁸ by the reduction of butyraldehyde with lithium aluminum hydride-H₄², and in 43% yield⁹ by improvements in the method described.

1-Butanol-H²-1-H₁² has been obtained⁷ by the reduction of butyraldehyde with deuterium and either a platinum or a nickel catalyst. With the latter catalyst, addition of HH² is apparently oriented to give predominantly 1-butanol-1-H₁². 2-Butanol-H²-2-H₁² has also been obtained⁶ from methyl ethyl ketone using the nickel catalyst.

2-Octanol-2-H² has also been prepared⁸⁻¹⁰ by the reduction of 2-octanone with sodium and acetic acid-H²

¹Organic Reactions, Vol. II, Wiley, New York, 1944, p. 400.

²W. Fickett, J. Am. Chem. Soc., 74, 4204 (1952).

³E. L. Eliel, *ibid.*, 71, 3970 (1949).

⁴E. R. Alexander, *ibid.*, 72, 3796 (1950).

⁵E. R. Alexander and A. G. Pinkus, *ibid.*, 71, 1786 (1949).

⁶W. v. E. Doering and R. W. Young, *ibid.*, 72, 631 (1950).

⁷L. C. Anderson and N. W. MacNaughton, *ibid.*, 64, 1456 (1942).

⁸A. R. Streitwieser, Jr., *ibid.*, 75, 5014 (1953).

⁹*Idem*, 77, 1117 (1955).

¹⁰D. G. Hill, W. A. Judge, P. S. Skell, S. W. Kantor and C. R. Hauser, *ibid.*, 74, 5599 (1952).

2-BROMOPENTANE-1,3-H₂



E. S. Lewis and C. E. Boozer, J. Am. Chem. Soc., 76, 791 (1954).

A. Procedure (Note 1)

2-Bromopentane-1,3-H₂², b.p. 116.5-117.0° (uncor.), is prepared in 80% yield by a modification¹ of the method of Hsueh and Marvel.² In a flask fitted with a mechanical stirrer, a thermometer and a dropping funnel, 1 mole of 2-pentanol-1,3-H₂² is cooled to -5°. With stirring, 1.1 moles of phosphorus tribromide is added dropwise, such that the temperature does

not exceed $+5^{\circ}$. The mixture is stored overnight and allowed to come to room temperature. To the mixture is slowly added about 300 ml. of cold water and then 150 ml. of ether. The ether solution is separated, washed with sodium carbonate solution and with water, and dried over calcium chloride.

After removal of ether, the product is distilled.

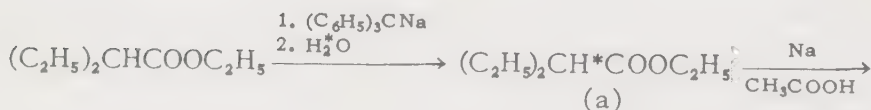
B. Notes

1. According to Cason and Mills,¹ conversion of a secondary alcohol to the corresponding bromide is likely to be accompanied by rearrangement of the intermediate carbonium ion, giving rise to a mixture of isomeric secondary bromides. They obtained experimental evidence which indicates that the reaction of bromine with the silver salt of a 2-alkyl-alkanoic acid yields an isomerically pure secondary alkyl halide.

¹J. Cason and R. H. Mills, J. Am. Chem. Soc., 73, 1354 (1951).

²C. Hsueh and C. S. Marvel, *ibid.*, 50, 855 (1928).

3-(BROMOMETHYL)PENTANE-3-H²



D. G. Hill, B. Stewart, S. W. Kantor, W. A. Judge and C. R. Hauser, J. Am. Chem. Soc., 76, 5129 (1954).

A. Procedure

(a) *Ethyl 2-Ethylbutyrate-2-H²*. To a solution of 0.89 mole of triphenylmethylsodium¹ in 1800 ml. of ether, under a nitrogen atmosphere in a 2-l. bottle with a standard tapered neck, is added 123 g. (0.85 mole) of ethyl 2-ethylbutyrate (Note 1). The mixture is kept at room temperature with occasional shaking for 24 hours (Note 2). Then, to the grey-colored mixture, still under nitrogen, is added 19 ml. (0.95 mole) of water-H₂². The mixture is shaken and set aside for 5 hours. The ether solution is decanted from the solid material, washed with cold water and dried over Drierite. The solvent is removed on a steam-bath, and the residue is cooled in an ice-bath. The liquid is decanted from solid triphenylmethane and distilled to obtain 81 g. (68%) of ethyl 2-ethylbutyrate-2-H², b.p. 150–152°.

(b) *2-Ethyl-1-butanol-2-H²*. To a stirred solution of 116 g. (0.75 mole) of ethyl 2-ethylbutyrate-2-H² in 2.5 l. of anhydrous ether, cooled with an ice-salt bath, is added gradually, during 2 hours, 230 g. (3.75 moles) of redistilled glacial acetic acid (b.p. 117°) and 83.6 g. (3.75 moles) of sodium. During the addition, the mixture is kept slightly acidic and is cooled below 10° (Note 3). The mixture is stirred for 60 hours and then shaken with cold water. The organic layer is separated, freed of ether and heated under reflux with 42 g. of potassium hydroxide in 270 ml. of methanol. Fractionation of the mixture yields 49 g. (53%) of 2-ethyl-1-butanol-2-H², b.p. 144-146° (Note 4).

(c) *3-(Bromomethyl)pentane-3-H²*. 2-Ethyl-1-butanol-2-H², 41 g. (0.4 mole), is treated with dry hydrogen bromide at 100° (Note 5). After the removal of unreacted alcohol by extraction with cold 95% sulfuric acid, water is added, and the mixture is made basic to phenolphthalein by the addition of ammonium hydroxide. The organic layer is dried over Drierite and distilled to obtain 43 g. (66%) of 3-(bromomethyl)pentane-3-H², b.p. 143-145, n_D^{25} 1.4495 (Notes 6 and 7).

B. Notes

1. This material had b.p. 150-151° and n_D^{25} 1.4014.
2. The deep-red color of the reagent is only partially discharged after 4 hours.
3. A little phenolphthalein powder was used as indicator.
4. The reported² boiling point of 2-ethyl-1-butanol is 147-147.6°.
5. See the procedure for the preparation of dodecyl bromide.³
6. Values reported² for 3-(bromomethyl)pentane are: b.p. 143-144° and n_D^{25} 1.4490.
7. The β -elimination reactions of (2-bromoethyl)benzene with sodium hydroxide-H² in methanol-H² and water-H₂ and of 1-bromo-2-ethylbutane-2-H² with potassium amide in liquid ammonia were shown to take place without accompanying hydrogen-deuterium exchange and deuterium-hydrogen exchange, respectively. These results support the bimolecular mechanism E2 in which the proton (or deuteron) and halide ion are eliminated simultaneously.

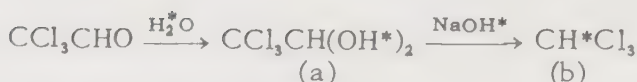
¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 607.

²H. A. Shonle, J. H. Waldo, A. K. Keltch and H. W. Coles, J. Am. Chem. Soc., 58, 585 (1936).

³*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 246.

TRICHLOROMETHANE- H^2 (Chloroform- H^2)

METHOD I



F. W. Breuer, J. Am. Chem. Soc., 57, 2236 (1935).

A. Procedure

(a) *Chloral Hydrate- H^2* . Chloral is distilled through a column (30 \times 0.9 cm.) packed with glass helices. The middle fraction is redistilled, and the vapors are passed over anhydrous calcium sulfate kept at 100°. After a third distillation, the middle fraction, n_D^{20} 1.4568, is used. To 14.72 g. (0.1 mole) of the purified chloral is added gradually, with cooling, 5.47 g. (0.22 mole) of water- H^2 (d_{20}^{20} 1.1079).

(b) *Trichloromethane- H^2* , (*Chloroform- H^2*). To a mixture of sodium hydroxide- H^2 and water- H^2 , prepared from 2.2 g. (0.096 g. atom) of sodium metal and 5 g. (0.25 mole) of water- H^2 (Note 1), in a flask equipped with a reflux condenser, is added the chloral hydrate- H^2 solution over a period of five hours. The flask is cooled with a bath kept below 5° (Note 2) during the addition and, after about 12 hours at room temperature, the reaction is gently warmed for 10 minutes. Separation of the reaction mixture into two layers is effected by centrifugation, and the chloroform- H^2 layer is separated, dried over freshly ignited calcium oxide, distilled and redistilled. The yield of chloroform- H^2 , having the following physical properties, is 7.85 g. (65.7%): m.p. range -64.69° to -64.15°; f.p. -64.12°; d_4^{20} 1.5004; n_D^{20} 1.4450 (Note 3).

METHOD II



R. C. Lord, B. Nolin and H. D. Stidham, J. Am. Chem. Soc., 77, 1365 (1955).

A. Procedure (Note 4)

A mixture of 15 g. of dry calcium carbonate and 16 g. of dry sodium carbonate (Note 5) is placed in a 0.5-l., three-necked flask fitted with two small dropping funnels and a Friedrich condenser. The upper opening of the condenser is connected to an apparatus similar to that of Earing and Cloke¹ for separation of the product (Note 6). The entire system is then dried and, while the flask is immersed in a bath at 110°, 28 g. of water- H^2 is added. This is followed by the dropwise addition of 48 g. of

trichloroacetyl chloride. Chloroform- H^2 soon forms and distills over into the trap. The temperature of the bath is gradually raised to about 160° as required by the rate of distillation of the product. After 2-3 hours the reaction is complete; the yield of chloroform- H^2 in the trap is 26 g. (81% based on acyl chloride).

The temperature of the bath is raised and slight vacuum is applied to recover unreacted water- H_2^2 (23 g.). After the chloroform- H^2 is dried and distilled, the yield is 76%; n_D^{25} 1.4428 (Note 7).

METHOD III



W. M. Boyer, R. B. Bernstein, T. L. Brown and V. H. Dibeler, J. Am. Chem. Soc., 73, 770 (1951).

A. Procedure

The preparation of pure chloroform- H^2 by the cleavage of trichloroacetophenone with sodium hydroxide- H^2 is according to the chloroform synthesis reported by Aston.² Using a vacuum apparatus, approximately 0.015 mole of α,α,α -trichloroacetophenone is added slowly, at -78° , to a solution of 0.015 g. atom of sodium in 0.3 mole of water- H_2^2 (Note 8). The chloroform- H^2 , which separates upon warming the mixture, is isolated, dried over Drierite and distilled through a small Vigreux column. The yield is about 30% (Note 9).

B. Notes

1. Details of the preparation of sodium hydroxide- H^2 are given by Breuer.
2. The first few drops of chloral cause a vigorous reaction, which then proceeds smoothly.
3. According to Breuer, the melting ranges of chloroform and chloroform- H^2 , prepared from chloral hydrate and chloral hydrate- H_2^2 , respectively, indicated that these compounds were not quite pure. However, the Raman spectrum of the chloroform- H^2 gave no evidence of the presence of ordinary chloroform. Boyer³ later made a study of the chloral reaction and reported from 3.16 to 13% of ordinary chloroform in the product. It was suggested that the hydrogen atom of chloral hydrate- H_2^2 appears in the product as ordinary chloroform, in part, but the results indicated that the transfer of hydrogen to the halogen-bearing carbon does not proceed by an exchange mechanism involving the solvent.
4. The following procedure is a modification of that described by Earing and Cloke¹ and obviates the time-consuming preparation of calcium trichloroacetate and the inconvenient use of metallic sodium.

5. When sodium carbonate is used alone the yield is about 60%.

6. The apparatus, shown diagrammatically by Earing and Cloke,¹ was similar to Natelson's modification⁴ of the moisture trap designed by Dean and Stark.⁵ The apparatus contained a minimum of joints and included a bulb, just above the reaction flask, to eliminate the effects of foaming which may occur toward the end of the reaction. Lord, *et al.*, replaced this bulb with a Friedrich condenser.

7. The infrared absorption spectrum indicated the product to be at least 98% chloroform-H².

8. This relatively large amount of water-H₂² was used to ensure that all the sodium benzoate remained in solution throughout the reaction.

9. The mass spectrum of this material indicated it to contain $0.8 \pm 0.1\%$ of ordinary chloroform.

C. Other Preparations

Chloroform-H² has been prepared by the reaction of sodium hydroxide-H² ^{3, 6-9, 12} and of calcium hydroxide-H₂² ^{10, 13} with chloral.

Bromoform-H² has been prepared^{3, 6, 11} by treating bromal with a solution of sodium hydroxide-H² in water-H₂². According to infrared and mass spectrometric analyses,^{3, 6} the product contained 4-5% of ordinary bromoform.

¹M. S. Earing and J. B. Cloke, *J. Am. Chem. Soc.*, **73**, 769 (1951).

²J. G. Aston, J. D. Newkirk, J. Dorsky and D. M. Jenkins, *ibid.*, **64**, 1415 (1942).

³W. M. Boyer, R. B. Bernstein, T. L. Brown and V. H. Dibeler, *ibid.*, **73**, 770 (1951).

⁴*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 37.

⁵E. W. Dean and D. D. Stark, *Ind. Eng. Chem.*, **12**, 486 (1920).

⁶G. P. Semeluk and R. B. Bernstein, *J. Am. Chem. Soc.*, **72**, 4830 (1950); *ibid.*, **76**, 3793 (1954).

⁷I. Lander and S. E. Wright, *Nature*, **158**, 381 (1946).

⁸J. Hine, R. C. Peek, Jr. and B. D. Oakes, *J. Am. Chem. Soc.*, **76**, 827 (1954).

⁹J. R. Madigan, F. F. Cleveland, W. M. Boyer and R. B. Bernstein, *J. Chem. Phys.*, **18**, 1081 (1950).

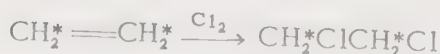
¹⁰R. Truchet and R. Lespiean, *Compt. rend.*, **202**, 1997 (1936).

¹¹W. M. Boyer and R. B. Bernstein, *J. Chem. Phys.*, **18**, 1073 (1950).

¹²C. M. Huggins and G. C. Pimentel, *ibid.*, **23**, 896 (1955).

¹³G. M. Barrow and E. A. Yerger, *J. Am. Chem. Soc.*, **76**, 5247 (1954).

1,2-DICHLOROETHANE-H₂²

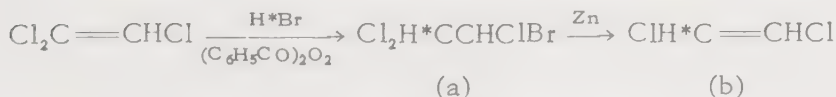


L. C. Leitch and A. T. Morse, *Can. J. Chem.*, **30**, 924 (1952).

Procedure

Using a vacuum system, 10 ml. of dichloromethane is placed in each of two spiral traps, which are then tared and cooled to -78° in Dry Ice-acetone baths. Slightly more than the theoretically required amount of chlorine to react with the ethylene- H_4^2 is then condensed in the traps. Ethylene- H_4^2 (8.0 liters) is bubbled through the chlorine solutions at such a rate that practically none escapes the second trap into a gas bottle containing bromine water (25 ml.). The reaction mixture is washed first with cold dilute sodium hydroxide solution and then with ice-water. After the solution is dried over anhydrous potassium carbonate, it is fractionated in a small column packed with glass helices. The yield of 1,2-dichloroethane- H_4^2 , b.p. 82° , is 28.6 g. (90%); d_4^{20} 1.3034, n_D^{20} 1.4420.

1,2-DICHLOROETHYLENE- H_1^2



L. C. Leitch and A. T. Morse, Can. J. Chem., 29, 1034 (1951).

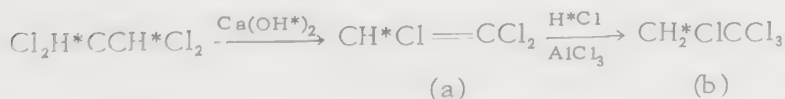
Procedure

(a) 2-Bromo-1,1,2-trichloroethane-1- H^2 . Hydrogen- H^2 bromide is made to add abnormally to trichloroethylene according to the method of Kharasch.¹ A sealed tube containing trichloroethylene, hydrogen- H^2 bromide and benzoyl peroxide is irradiated for 42 hours with ultraviolet light. A nearly quantitative yield of 2-bromo-1,1,2-trichloroethane-1- H^2 , b.p. $70-72^{\circ}$ (22 mm.), n_D^{20} 1.5295, is obtained.

(b) 1,2-Dichloroethylene- H_1^2 . *cis*- and *trans*-1,2-Dichloroethylene- H_1^2 are obtained by heating 2-bromo-1,1,2-trichloroethane-1- H^2 in ethanol with zinc dust. The isomers are separated by fractionation in a column packed with glass helices and finally distilled on a vacuum line.

¹M. S. Kharasch, J. A. Norton and F. R. Mayo, J. Org. Chem., 3, 48 (1939).

1,1,1,2-TETRACHLOROETHANE- H_2^2



L. C. Leitch and H. J. Bernstein, Can. J. Research, 28B, 35 (1950); H. J. Bernstein, *ibid.*, 28B, 132 (1950).

A. Procedure

(a) *Trichloroethylene-H²*. This compound is prepared by the reaction of a water-H₂² solution of calcium hydroxide-H₂² and 1,1,2,2-tetrachloroethane-H₂² (Note 1).

(b) *1,1,1,2-Tetrachloroethane-H²*. Dry hydrogen-H² chloride is added to trichloroethylene-H² in the presence of aluminum chloride (Note 2).

B. Notes

1. No details of the preparation are given in the original literature. Calcium hydroxide cannot be used in the reaction since hydrogen exchange with the trichloroethylene-H² occurs. The infrared absorption spectrum of the product indicated it to be nearly pure.

2. No further details of the synthesis are given by Bernstein. According to the infrared spectrum, the tetrachloro compound was practically pure.

1,1,2,2-TETRACHLOROETHANE-H₂²

F. W. Breuer, J. Am. Chem. Soc., 58, 1289 (1936).

A. Procedure (Note 1)

The all-glass apparatus (Figure XVI, 9) includes an acetylene generating system (Note 2), a chlorine purifying train (Note 3) and two catalyst-containing chlorination tubes (Note 4).

For the chlorination process, the first of the two catalyst tubes is illuminated with a 100-watt incandescent bulb, with metal reflector, at a distance of approximately 40 cm. The second catalyst tube is heated slightly with the flame of a wing-top burner. Purified chlorine is passed through the catalyst tubes at a rate of 16 ml. per minute prior to admission of the acetylene-H₂², from 8 g. (0.013 mole) of calcium carbide and 4 ml. (0.22 mole) of water-H₂², at a rate of 4 ml. per minute. Combination of the gases starts immediately as evidenced by the warming of the first tube and the formation of a liquid product. Very little gas passes through the terminal bubble counter until the acetylene is all consumed. The product is collected in a cold trap, maintained at -10°, between the two catalyst tubes. No product is formed in the second tube, and all the product is expelled from the first tube by heating it with the chlorine still flowing. The yield of tetrachloroethane-H₂², based on water-H₂², is 14.5 g. (78%).

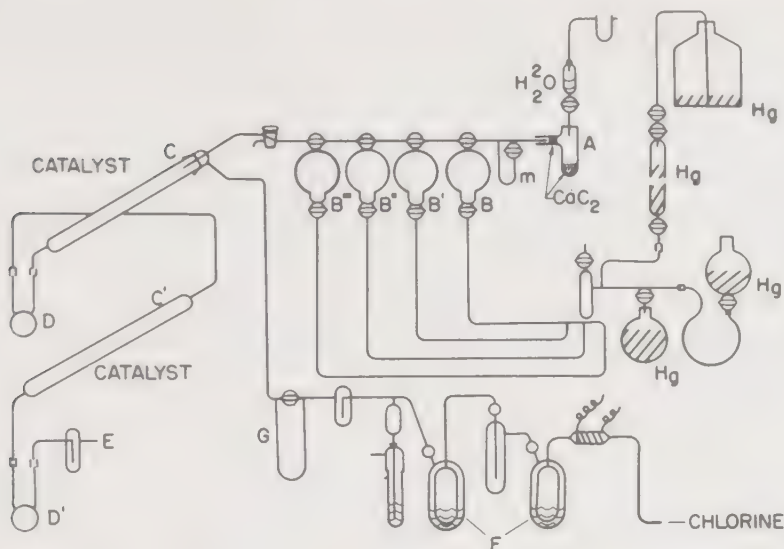


Fig. XVI, 9 Apparatus for the preparation of 1,1,2,2-tetrachloroethane- H_2^2 (F. W. Breuer). A, Acetylene generator; B-B''', acetylene storage bulbs; C-C', catalyst tubes; D-D', cold traps for collecting product; E, bubble counter; F, chlorine purifying train; G, flow meter.

For purification, the product is transferred to the boiling flask of a partial reflux-type fractionating column (length of packed section 37 cm., inner diameter 0.7 cm.). Using a reflux ratio of 10:1 at 60 mm., two fractions boiling at $68.8\text{--}70.7^\circ$ and 70.7° are collected (Note 5).

B. Notes

1. Most of the methods described in the literature were tested for adaptability to small-scale preparations, with the least risk of explosions. Bernstein¹ also reported the preparation of tetrachloroethane- H_2^2 by the reaction of Cl_2 and acetylene- H_2^2 . No details were given.

2. Acetylene- H_2^2 was generated from calcium carbide and water- H_2^2 and stored in four 500-ml. flasks interconnected by 3-way stopcocks and using redistilled mercury as confining liquid.

3. The all-glass chlorine purifying train consisted of: a platinized asbestos-manganese dioxide catalyst tube, heated electrically to 400° ; gas wash bottles containing saturated potassium permanganate solution and concentrated sulfuric acid, respectively; a pressure regulator for the adjustment of the gas velocity; and a capillary flow control with bubble counter.

4. The catalyst was prepared from a mixture of 7.5 parts of ferric oxide, 7.5 parts of reduced iron, 15 parts of aluminum oxide, 5.5 parts of antimony metal (powdered) and 15 parts of clean quartz sand. These components, which had been either ignited or dried *in vacuo* at 110° ,

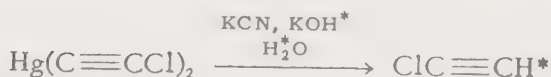
were mixed, suspended on ignited asbestos and packed rather loosely in the reaction tubes of heavy-walled (bomb) tubing, 15 mm. in diameter and 15 and 30 cm. in length, respectively.

5. The latter fraction was refractionated at 89 mm. to obtain three samples with boiling ranges of 77.5–79.5°, 79.5–80° and 80°. The following physical data were obtained, by a technique described earlier,² on the middle fraction of 8.5 g.: d_4^{20} 1.6118, n_D^{20} 1.4924 and b.p. 145.7 ± 0.05° (737 mm.).

¹H. J. Bernstein, Can. J. Research, 28B, 132 (1950).

²F. W. Breuer, J. Am. Chem. Soc., 57, 2236 (1935).

CHLOROACETYLENE-H²



A. A. Westenberg, J. H. Goldstein and E. B. Wilson, Jr., J. Chem. Phys., 17, 1319 (1949).

A. Procedure

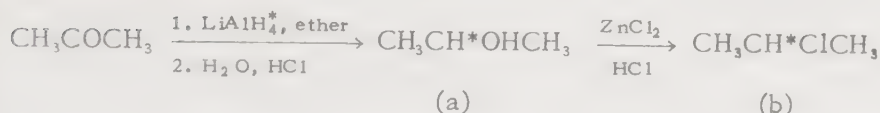
Chloroacetylene-H² is prepared by an adaptation of the procedure of Bashford.¹ In an all-glass system, with a stream of nitrogen flowing (Note 1), mercury chloroacetylde, 0.4 g., is reacted with a solution of 1.3 g. of potassium cyanide and 0.3 g. of potassium hydroxide-H² in 4.5 ml. of water-H₂² (Note 2). The chloroacetylene-H² is swept by the stream of nitrogen into a trap cooled with liquid nitrogen. Purification is accomplished by several bulb-to-bulb distillations under a good vacuum. Finally, high-boiling impurities are retained in a trap cooled with a Dry Ice-acetone bath at -80°.

B. Notes

1. The nitrogen is freed of oxygen by passage through a scrubber containing pyrogallol-potassium hydroxide reagent.

2. Since chloroacetylene is spontaneously combustible, it is necessary that all oxygen be excluded and it is recommended that only very small amounts of materials be used. It is especially important to evacuate the chloroacetylene receiver with a good vacuum system (oil pump and diffusion pump), while the receiver is immersed in liquid nitrogen, before allowing the product to warm to room temperature.

¹L. A. Bashford, H. J. Emelëus and H. V. A. Briscoe, J. Chem. Soc., 1938, 1358.

2-CHLOROPROPANE-2-H²

F. E. Condon, H. L. McMurry and V. Thornton, *J. Chem. Phys.*, **19**, 1010 (1951).

A. Procedure

(a) *2-Propanol-2-H²* (Note 1). In a 3-necked flask equipped with a reflux condenser protected by a drying tube, and a mercury-sealed stirrer, 0.7 mole of anhydrous acetone dissolved in ether is added to an ether solution of lithium aluminum hydride-H₄² in slight excess (Note 2). The addition is made at such a rate that the capacity of the reflux condenser is not exceeded (Note 3). The mixture is stirred for 2 hours following the addition. Then the reaction mixture is cooled in an ice-bath, and water is added dropwise until hydrogen is no longer evolved. Finally, hydrochloric acid is added until the precipitated aluminum hydroxide is dissolved. The 2-propanol-2-H² is isolated by fractional distillation, dried and redistilled (Note 4).

(b) *2-Chloropropane-2-H²*. Dry 2-propanol-2-H² is treated with zinc chloride and concentrated hydrochloric acid. The mixture is then fractionated to obtain the 2-chloropropane-2-H².

B. Notes

1. For general information on lithium aluminum hydride reductions, see *Organic Reactions*.¹
2. Lithium aluminum hydride-H₄² is available from Metal Hydrides Inc., Beverly, Mass.
3. The operation is conducted in a hood with a good draft.
4. Williams² has prepared 2-propanol-2-H² by the same method using lithium aluminum hydride-H₄². The 2-propanol-2-H² was isolated by a 12-hour continuous ether extraction after the addition of nonisotopic isopropyl alcohol.

C. Other Preparations

2-Propanol-2-H² has been obtained^{3,4} by the treatment of 2-propanol-H²-2-H² with ordinary water to exchange hydroxyl hydrogen-H² with hydrogen.

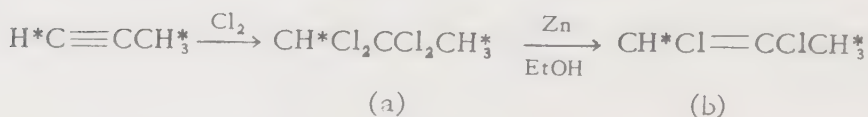
¹*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 469.

²E. D. Williams, K. A. Krieger and A. R. Day, *J. Am. Chem. Soc.*, **75**, 2404 (1953).

³F. H. Westheimer and N. Nicolaides, *ibid.*, 71, 25 (1949).

⁴L. Friedman and J. Turkevich, *ibid.*, 74, 1669 (1952).

cis- AND *trans*-1,2-DICHLOROPROPENE- H_4^2



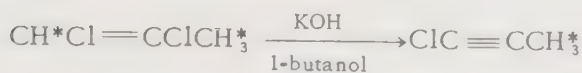
L. C. Leitch, *Can. J. Chem.*, 31, 385 (1953).

A. Procedure

(a) *1,1,2,2-Tetrachloropropane- H_4^2* . The apparatus used is similar to that described by Taylor and Morey;¹ the reactor (2 liters) is heated by means of an infrared lamp at a distance of six inches. Dry chlorine and propyne- H_4^2 , in the ratio of 2:1, are introduced into the reactor through flow meters. After a short induction period, during which the temperature of the gas mixture rises to 60–70°, the reaction starts and liquid product is evident. When 17.0 l. of propyne- H_4^2 has been introduced at the rate of 2 l. per hour, the liquid product is withdrawn, washed with aqueous potassium carbonate and dried over anhydrous potassium carbonate. The yield of crude product is 107 g. On distillation of the crude product at atmospheric pressure through a Stedman column (12 × 0.75 inches), a fraction of boiling range 62 to 98° is collected. This product, on re-distillation in a smaller column, is mainly *trans*-1,2-dichloropropene- H_4^2 , b.p. 75°, n_D^{20} 1.4479; yield, 15.0 g. (20%). The residual 1,1,2,2-tetrachloropropane- H_4^2 is fractionated under reduced pressure. After a forerun of 5.5 ml. boiling up to 79° (64 mm.), the main product, 82.5 g. (63%), n_D^{20} 1.4848, distills at 81° (64 mm.).

(b) *cis- and trans-1,2-Dichloropropene- H_4^2* . In a 1-liter, 3-necked flask equipped with a stirrer and reflux condenser, 37.5 g. of 1,1,2,2-tetrachloropropane- H_4^2 is added gradually to a boiling suspension of zinc dust (20 g.) in 200 ml. of ethanol. Any allene- H_4^2 formed from the isomeric 1,2,2,3-tetrachloropropane- H_4^2 is condensed in a trap at -78°. The nearly quantitative yield of product is separated into the *cis*- and *trans*-isomers boiling at 92° and 75°, respectively, by distillation through a Stedman column. Refractive indices of *cis*- and *trans*-1,2-dichloropropene- H_4^2 are, respectively, n_D^{20} 1.4528 and n_D^{20} 1.4479.

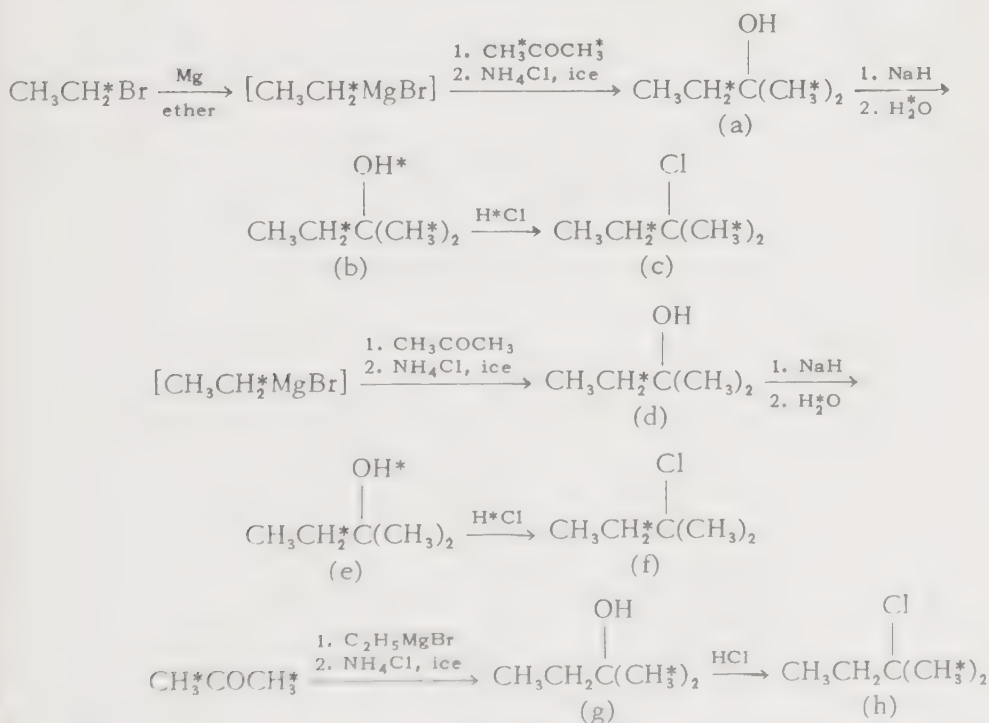
¹R. F. Taylor and G. H. Morey, *Ind. Eng. Chem.*, 40, 432 (1948).

1-CHLOROPROPYNE-H₃²

A. T. Morse and L. C. Leitch, Can. J. Chem., 32, 500 (1954).

Procedure

A solution of 36.4 g. of *cis*-1,2-dichloropropene-H₄² in 20 ml. of 1-butanol is added dropwise to a stirred refluxing solution of 25 g. of potassium hydroxide in 300 ml. of 1-butanol. Loss of the volatile 1-chloropropyne-H₃² is avoided by circulating water at 5° through the reflux condenser. After addition of the halide is complete, the reaction mixture is cooled to 0°, and the reflux condenser is replaced by a 12-inch Stedman column and a still-head. The product distills as an azeotrope, b.p. 31–32°. The condensate is freed of alcohol by distillation *in vacuo* through a U-tube containing phosphorus pentoxide. After the product is redistilled through the Stedman column, the yield of 1-chloropropyne-H₃², b.p. 31.7° (750 mm.), is 19.4 g. (78.5%); n_D^{20} 1.4105, 98.17 atom per cent H².

2-CHLORO-2-METHYL-H₃²-BUTANE-1,3-H₅²

V. J. Shiner, Jr., J. Am. Chem. Soc., 75, 2925 (1953).

A. Procedure

(a) *2-Methyl-H₃²-2-butanol-1,3-H₅²*. A solution of 7 g. (0.064 mole) of 1-bromoethane-1-H₂² in 15 ml. of ether is added slowly to 1.5 g. (0.062 mole) of magnesium and 15 ml. of dry ether in a 50-ml. 3-necked flask. After the ethylmagnesium-1-H₂² bromide solution stands for several hours, 4.0 g. (0.069 mole) of 2-propanone-H₆² is added dropwise. The reaction mixture is kept overnight and then poured onto an excess of ammonium chloride and ice. The ether layer and ether extracts of the water layer are combined, and the ether is removed through a helix-packed column. The product, purified by distillation, weighs 3.6 g. (0.041 mole, 66%).

(b) *2-Methyl-H₃²-2-butanol-H²-1,3-H₅²*. 2-Methyl-H₃²-2-butanol-1,3-H₅², 3.6 g. (0.041 mole), is refluxed in ether with a slight excess of sodium hydride for 24 hours. After the ether is removed, the remaining sodium alcoholate is hydrolyzed with 1.5 g. of water-H₂². The 2-methyl-H₃²-2-butanol-H²-1,3-H₅² and excess water-H₂² are distilled under vacuum and collected in a Dry Ice-cooled flask.

(c) *2-Chloro-2-methyl-H₃²-butane-1,3-H₅²*. Dry hydrogen-H² chloride is distilled into the flask with the above mixture of 2-methyl-H₃²-butanol-H²-1,3-H₅² and water-H₂², which is then warmed. The two layers which form are separated, and the organic layer, after washing once with aqueous sodium bicarbonate, is dried over anhydrous potassium carbonate and distilled. The yield of 2-chloro-2-methyl-H₃²-butane-1,3-H₅² is 3.0 g. (0.029 mole), 70%.

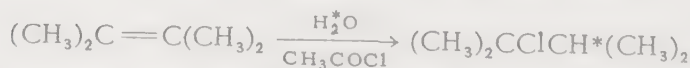
(d) *2-Methyl-2-butanol-3-H₂²*. This alcohol is synthesized, according to the above procedure for 2-methyl-H₃²-2-butanol-1,3-H₅², using ethylmagnesium-1-H₂² bromide and ordinary acetone.

(e) *2-Methyl-2-butanol-H²-3-H₂²*. As in the preparation of 2-methyl-H₃²-2-butanol-H²-1,3-H₅², 2-methyl-2-butanol-3-H₂² is treated with sodium hydride and water-H₂².

(f) *2-Chloro-2-methylbutane-3-H₂²*. 2-Methyl-2-butanol-H²-3-H₂² is treated with hydrogen-H² chloride as described under (c) above.

(g) *2-Methyl-H₃²-2-butanol-1-H₃²*. Ethylmagnesium bromide is treated with 2-propanone-H₆² as in the preparation of (a) above.

(h) *2-Chloro-2-methyl-H₃²-butane-1-H₃²*. 2-Methyl-H₃²-2-butanol-1-H₃² is treated with concentrated hydrochloric acid without first forming the hydroxy-H² alcohol. The product is isolated as described for (c) above.

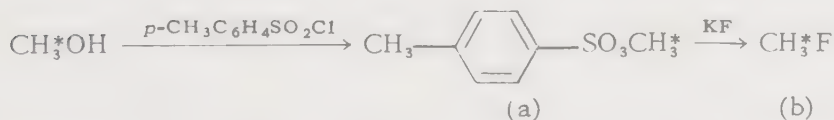
2-CHLORO-2,3-DIMETHYLBUTANE-3-H²

V. J. Shiner, Jr., J. Am. Chem. Soc., 76, 1603 (1954).

Procedure

During a period of 48 hours, 2.0 g. (0.10 mole) of water- H_2^2 is slowly added dropwise to a solution of 7.3 g. (0.087 mole) of 2,3-dimethyl-2-butene in 22.0 g. (0.28 mole) of acetyl chloride. After the addition is complete, the solution is refluxed for 2 hours and poured onto ice. The product is extracted with ether and dried over potassium carbonate. After removal of ether, distillation of the product yields 7.5 g. (0.062 mole, 72%) of 2-chloro-2,3-dimethylbutane-3- H^2 ; b.p. $109.5\text{--}110^\circ$ (740 mm.), n_D^{25} 1.4182.

FLUOROMETHANE- H_3^2



W. F. Edgell and L. Parts, J. Am. Chem. Soc., 77, 5515 (1955).

A. Procedure

(a) *Methyl- H_3^2 p-Toluenesulfonate*. This ester is prepared essentially according to the procedure of Edgell and Parts.¹ To a mixture of 15.7 g. (0.448 mole) of methyl- H_3^2 alcohol and 94.0 g. (0.493 mole) of *p*-toluenesulfonyl chloride at -44° is added 443 g. (5.6 moles) of pyridine, at a constant rate with rapid stirring, over a period of 2 hours. The reaction is allowed to proceed for an additional hour and then is quenched by pouring the mixture into 600 ml. of ice-water.

Excess *p*-toluenesulfonyl chloride is hydrolyzed by shaking the resulting suspension for 5 minutes. The suspension is extracted with about 340 ml. of ether, and dilute sulfuric acid, containing finely crushed ice, is added to the separatory funnel to neutralize the excess of pyridine. The aqueous layer is removed, and the ether solution is washed successively with: ice-cold dilute sulfuric acid, ice-water, ice-cold dilute potassium hydroxide and again with ice-water. The ester solution is dried over sodium carbonate, and removal of the ether leaves 69.9 g. (82.4%) of methyl- H_3^2 *p*-toluenesulfonate; n_D^{20} 1.5179 (Note 1).

(b) *Fluoromethane- H_3^2* . To 69.9 g. of methyl- H_3^2 *p*-toluenesulfonate is added 64.3 g. (1.11 moles) of anhydrous potassium fluoride. Without added solvent,¹ the pressure in the flask, which is connected to a liquid nitrogen-cooled trap, is reduced to approximately 500 mm. The temperature of the mixture is raised to 250° during 2 hours and maintained at that point for 5 hours. The yield of product, collected in the liquid nitrogen trap, is 11.0 g. (80.5%) (Note 2). The product is purified by distilla-

tion through a Podbielniak Heli-Robot column. Assuming that the boiling point of methyl fluoride is -78.414° ,² that of methyl- H_3^2 fluoride is -77.9° .

B. Notes

1. By analyzing the melting curve of their unlabeled methyl *p*-toluene-sulfonate, Edgell and Parts¹ estimated the m.p. to be 24.13° and the purity to be 95.0 ± 2.5 mole per cent. The reported melting point of methyl tosylate is 28° .³

2. The product contained about 5% chloromethane- H_3^2 , presumably due to the presence of some *p*-toluenesulfonyl chloride in the methyl- H_3^2 ester.

¹W. F. Edgell and L. Parts, J. Am. Chem. Soc., 77, 4899 (1955).

²A. Michels and T. Wassenaar, Physica, 14, 104 (1948).

³F. Drahowzal and D. Klamann, Monatsh., 82, 452 (1951).

TRIFLUOROMETHANE- H^2 (Fluoroform- H^2)



S. R. Polo and M. K. Wilson, J. Chem. Phys., 21, 1129 (1953).

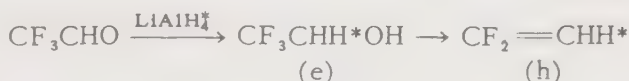
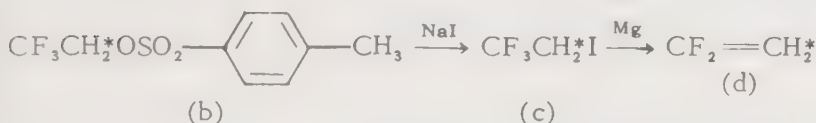
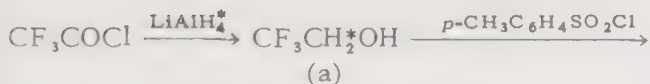
A. Procedure

About 4 g. of trifluoroiodomethane is distilled *in vacuo* and condensed at -80° in a stainless-steel bomb containing 3 g. of yellow phosphorus. The bomb is then heated at 250° for 36 hours.¹ The reaction mixture is fractionated under vacuum into a more volatile portion consisting of unreacted trifluoroiodomethane, a middle fraction consisting mainly of tris(trifluoromethyl)phosphine, b.p. 17.3° (760 mm.),¹ and a final portion consisting of a mixture of bis(trifluoromethyl)iodophosphine, b.p. 73° (755 mm.),¹ and trifluoromethyldiiodophosphine, b.p. 133° (413 mm.). This latter portion is hydrolyzed with a 50% solution of sodium hydroxide- H^2 in water- H_2^2 to obtain fluoroform- H^2 (Note 1). Traces of unreacted phosphines are easily separated from the product by bulb-to-bulb distillation *in vacuo*.

B. Notes

1. According to Bennett,¹ tris(trifluoromethyl)phosphine is hydrolyzed quantitatively to fluoroform, also.

¹F. W. Bennett, G. R. A. Brandt, H. J. Emeléus and R. N. Haszeldine, Nature, 166, 225 (1950).

1,1-DIFLUOROETHYLENE- H_2^2 

W. F. Edgell and C. J. Ultee, J. Chem. Phys., 22, 1983 (1954).

A. Procedure

(a) *2,2,2-Trifluoroethanol-1- H_2^2* . This compound is prepared according to the following procedure of Henne¹ by substituting lithium aluminum hydride- H_2^2 for the reducing agent. A 5-liter, 3-necked flask, equipped with a Dry Ice-type reflux condenser, a sealed stirrer and a gas inlet tube, is dried with a stream of dry nitrogen as it is flamed. Into a stirred mixture of 54 g. (1.42 moles) of lithium aluminum hydride and 3 liters of dry ether in the flask is introduced trifluoroacetyl chloride as fast as the return from the reflux condenser permits, until 350 g. is added (Note 1). Then the mixture is refluxed for 1 hour more (Note 2). The inlet tube is replaced by a dropping funnel, and 200 ml. of water is slowly added (Note 3). The resulting clear ether solution is decanted from the white precipitate into 1500 ml. of 6 N sulfuric acid containing ice. The ether layer is separated and used in three portions for extraction of the aqueous layer. The latter is poured back into the reaction flask with the solid residue and further extracted with fresh ether. Fractionation of the combined ether extracts affords 285 g. of material boiling at 74–75° (Note 4), which is then distilled from concentrated sulfuric acid. The yield of 2,2,2-trifluoroethanol, b.p. 74°, is 85%.

(b) *2,2,2-Trifluoroethyl- H_2^2 p-Toluenesulfonate*. In a 1-liter flask equipped with a reflux condenser, a stirrer and a dropping funnel, 50 g. (0.5 mole) of 2,2,2-trifluoroethanol-1- H_2^2 is mixed with 105 g. (0.55 mole) of *p*-toluenesulfonyl chloride. The mixture is cooled in an ice-bath, and 80 g. of pyridine is slowly added. The resulting mixture is maintained at 0° and stirred overnight; then it is poured onto ice and neutralized with dilute hydrochloric acid. The ester, which is collected on a filter and washed with water, is recrystallized from petroleum ether and dried under vacuum. The yield of ester, m.p. 40.5°, is 115 g. (90%).

(c) *1,1,1-Trifluoro-2-iodoethane-H₂²*. In a 1-liter flask, equipped with a short fractionating column, are mixed 100 g. (0.40 mole) of 2,2,2-trifluoroethyl-H₂² *p*-toluenesulfonate, 75 g. (0.5 mole) of sodium iodide and 300 ml. of bis(2-hydroxyethyl) ether. The mixture is heated to 100–150°, and 1,1,1-trifluoro-2-iodoethane-H₂² distills at 52°. After the crude product is washed with water and dried over Drierite, the yield is 75–80 g. (about 90%).

(d) *1,1-Difluoroethylene-H₂²* (Note 5). A solution of 50 g. (~ 0.2 mole) of 1,1,1-trifluoro-2-iodoethane-H₂² is mixed with an equal volume of ether and dried over Drierite. In a dry 200-ml. 3-necked flask, equipped with a stirrer, a dropping funnel and a Dry Ice-trichloroethylene condenser, are placed 10 g. of magnesium (Note 6) and a small crystal of iodine. The flask is warmed to disperse the iodine and, after it has cooled, 10 ml. of dry ether is added. The dry solution of 1,1,1-trifluoro-2-iodoethane in ether is then introduced dropwise with stirring (Note 7). Finally the 1,1-difluoroethylene-H₂² is distilled into a receiver cooled with liquid nitrogen. After repeated distillation of the product, the yield is 75%.

(e) *2,2,2-Trifluoroethanol-1-H₂²*. To 1 liter of dry ether in a 2-liter 3-necked flask, equipped with a gas inlet tube, a stirrer and a Dry Ice-type condenser, is added 9 g. (0.25 mole) of lithium aluminum hydride-H₄². Into this stirred mixture is slowly passed 100 g. (about 1 mole) of 2,2,2-trifluoroacetaldehyde (Note 8). After the aldehyde is added, the excess hydride is carefully decomposed with water. The resulting slurry is poured into a mixture of 800 g. of ice and 50 ml. of concentrated sulfuric acid. The ether layer is separated, and the water layer is extracted once with additional ether. Fractionation of the ether solution yields 80 g. (81%) of 2,2,2-trifluoroethanol-1-H₂², b.p. 75°.

(f) *2,2,2-Trifluoroethyl-1-H₂²* *p*-Toluenesulfonate, (g) *1,1,1-Trifluoro-2-iodoethane-H₂²* and (h) *1,1-Difluoroethylene-H₂²* are prepared according to the procedures described above.

B. Notes

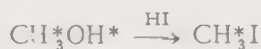
1. The addition of 350 g. (2.64 moles) took about 3.5 hours.
2. An electric heating mantle was used.
3. Addition of water to a lithium aluminum hydride solution should be done with extreme care.
4. This is 2,2,2-trifluoroethanol containing 5–10% of water.
5. This compound was prepared by adaptation of the method of Gilman and Jones.²
6. The magnesium was washed and dried.
7. No heating is necessary to start or maintain the reaction.
8. Trifluoroacetaldehyde was prepared from trifluoroacetic acid by reduction with lithium aluminum hydride according to the procedure of Husted and Ahlbrecht.³

¹A. L. Henne, R. M. Alm and M. Smook, J. Am. Chem. Soc., 70, 1968 (1948).

²H. G. Gilman and R. G. Jones, *Ibid.*, 65, 2037 (1943).

³D. R. Husted and A. H. Ahlbrecht, *ibid.*, 74, 5422 (1952).

IODOMETHANE-H₃²



J. Beersmans and J. C. Jungers, Bull. soc. chim. Belges, 56, 238 (1947).

A. Procedure

(a) *Iodomethane-H₃²*. In an all-glass vacuum apparatus (Figure XVI, 10) hydrogen iodide is generated by the action of water, added slowly, on phosphorus triiodide. The gaseous hydrogen iodide passes through a cold trap and into the reaction flask containing methanol-H₄² (Note 1). The reaction flask is attached to a water-condenser which is itself surmounted by a Dry Ice-type of condenser containing alcohol. By the addition of Dry Ice chips to the alcohol, the temperature of the top condenser is adjusted to permit the escape of the vapors of methyl iodide and some of the excess hydrogen iodide. The mixture of gases is then passed through two scrubbers containing, respectively, water and sodium hydroxide solution. The methyl-H₃² iodide and some entrained water vapor are then collected in a trap cooled with liquid nitrogen. The methyl-H₃² iodide is transferred, under vacuum, through a phosphorus pentoxide drying tube, into the reservoir-flask of an all-glass vacuum-jacketed column provided with a low-temperature reflux head (Note 2). By the proper adjustment of the temperatures of the reservoir-flask and the low-temperature head, the methyl-H₃² iodide is fractionated and collected in evacuated bulbs.

(b) *Chloromethane-H₃²*. Using the same apparatus, chloromethane-H₃² is prepared from methyl-H₃² alcohol-H² and hydrogen chloride with anhydrous zinc chloride catalyst.

B. Notes

1. The hydrogen iodide is present in great excess.
2. This is a Dry Ice-type of condenser constructed around the upper portion of the column.

C. Other Preparations

Iodomethane-H₃² has been prepared by the reaction of methanol-H₄², phosphorus and iodine,^{1,2} and in 94% yield by treatment of bromomethane-H₃² with calcium iodide at 75°.³ Iodomethane-C¹⁴-H₃² has been prepared⁴ from methanol-C¹⁴-1-H₃², phosphorus and iodine.

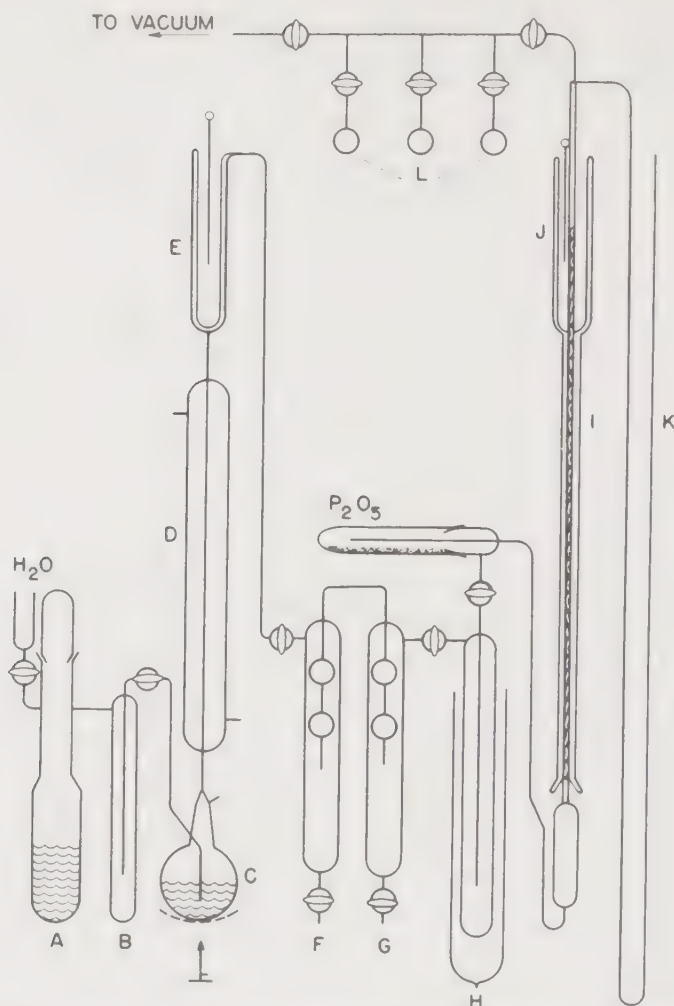


Fig. XVI, 10 Apparatus for the synthesis and purification of methyl- H_3 halides (J. Beersmans and J. C. Jungers). A, phosphorus triiodide container; B, cold trap; C, reaction flask containing methanol- H_3 ; D, water-cooled condenser; E, Dry Ice-type condenser; F, water scrubber; G, sodium hydroxide scrubber; H, cold trap for collecting crude product; I, vacuum-jacketed column; J, low-temperature reflux head; K, manometer; L, product bulbs.

Chloromethane- H_3 has been prepared⁵ by the action of aluminum chloride on methyl- H_3 alcohol, according to the procedure of Norris and Sturgis;⁶ and by the reaction of *N*-methyl- H_3 -benzamide with phosphorus pentachloride.⁷

¹V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, *J. Biol. Chem.*, **140**, 625 (1941).

²N. Solimene and B. P. Dailey, *J. Chem. Phys.*, **23**, 124 (1955).

³B. Nolin, *Can. J. Chem.*, **32**, 1 (1954).

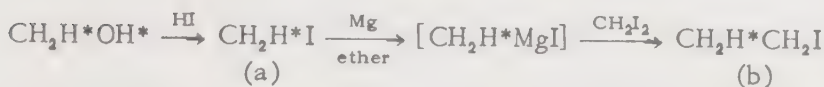
⁴J. R. Rachele, E. J. Kuchinskas, F. H. Kratzer and V. du Vigneaud, *J. Biol. Chem.*, **215**, 593 (1955).

⁵G. Matlock, G. Glockler, D. R. Bianco and A. Roberts, J. Chem. Phys., 18, 332 (1950).

⁶J. F. Norris and B. M. Sturgis, J. Am. Chem. Soc., 61, 1413 (1939).

⁷H. D. Noether, J. Chem. Phys., 10, 664 (1942).

1-iodoethane-2-H₁²



A. Langseth and B. Bak, Kg. Danske Videnskab. Selskab, Mat.-fys. Medd., 24, No. 3 (1947).

A. Procedure

(a) *Iodomethane-H₁²*. A mixture of 0.5 mole of methanol-H²-1-H₁² and an excess of constant boiling hydriodic acid is heated under reflux. Iodomethane-H₁² is isolated by distillation and dried; yield 60%.

(b) *1-Iodoethane-2-H₁²*. Methylmagnesium-H₁² iodide reagent, prepared from the above iodide, is immediately added dropwise to an ethereal solution of methylene iodide (Note 1) at 10–20° (Note 2). The resulting 1-iodoethane-2-H₁² is isolated and fractionated through a 60-cm. column. The yield of product, b.p. 71–75°, is 25%.

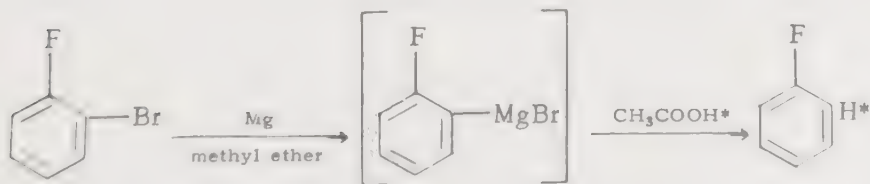
B. Notes

1. The corresponding reaction with methylene bromide is not feasible.
2. Considerable heat is evolved during the reaction.

C. Other Preparations

Langseth and Bak also prepared 1-iodoethane-2-H₁² by a similar procedure using bromomethane-H₁².

1-FLUOROBENZENE-2-H²



G. E. Hall, R. Piccolini and J. D. Roberts, J. Am. Chem. Soc., 77, 4540 (1955).

A. Procedure (Note 1)

1-Bromo-2-fluorobenzene, 61.3 g. (0.350 mole), is dissolved in 60 ml. of dry ethyl ether. To start the reaction, a piece of magnesium is added to 1 ml. of the ether solution. This mixture is added to 8.50 g. (0.350 g. atom) of magnesium in a 500-ml. 3-necked flask equipped with a stirrer, a Dry-Ice condenser protected with a drying tube, a dropping funnel and a gas inlet. Gaseous methyl ether, dried by passage through a Drierite-filled tower, is run into the flask until 50 ml. of liquid has condensed. The ethyl ether solution of 1-bromo-2-fluorobenzene is then added dropwise during 100 minutes. After the mixture is stirred for an additional 60 minutes, a solution of 26.2 g. (0.430 mole) of acetic acid- H^2 in ethyl ether is added dropwise with vigorous stirring (Note 2). When this addition is complete, the methyl ether is evaporated, and the residue is extracted with ethyl ether (Note 2). The ether solution is separated, washed with dilute acid and water, and dried. After removal of ether, fractionation of the residual mixture gives 9.7 g. of starting material, b.p. $154-158^\circ$ (745 mm.), n_D^{25} 1.5291. The yield of 1-fluorobenzene-2- H^2 , b.p. $83.0-85.0^\circ$ (745 mm.), is 18.8 g. (55% conversion, 66% yield).

Other deuterium-labeled substituted benzenes, which were prepared by a similar procedure in ethyl ether solvent, are given in the following table.

TABLE XVI,9
Deuterium-labeled Substituted Benzenes

Product*	B.P., $^\circ C$	n_D^{25}
1-Fluorobenzene-3- H^2	82.5-83.5 (744 mm.)	1.4616
1-Fluorobenzene-4- H^2	82.3-84.0 (752 mm.)	1.4612
α, α, α -Trifluorotoluene-2- H^2	99.8-100.5 (742 mm.)	1.4113
α, α, α -Trifluorotoluene-3- H^2	100.0-101.2 (738 mm.)	1.4120
α, α, α -Trifluorotoluene-4- H^2	100.1-100.5 (740 mm.)	1.4118
Anisole-2- H^2	149.0-150.5 (742 mm.)	1.5137
Anisole-3- H^2	149.0-150.0 (743 mm.)	1.5138
Anisole-4- H^2	149.0-150.0 (742 mm.)	1.5133
Toluene-2- H^2	108.9-109.0 (740 mm.)	1.4938
Benzene- H_1^2	79.0-79.0 (741 mm.)	1.4954

*Infrared absorption data for these compounds are presented by Hall, *et al.*

B. Notes

1. 1-Bromo-2-fluorobenzene and magnesium were found to react smoothly in ethyl ether; however, as reported,¹ the reaction product was not the expected Grignard reagent. 2-Fluorophenylmagnesium bromide was successfully prepared in methyl ether solvent.

2. Acetic acid- H^2 was preferred to water- H_2^2 for this decomposition of the Grignard reagent because it is more economical of deuterium, and its ether solubility permits better control of the reaction than is possible with water- H_2^2 .

¹M. S. Kharasch, H. Pines and J. H. Levine, *J. Org. Chem.*, 3, 347 (1938).

NITROETHANE-1- H_1^2



S. H. Maron and V. K. LaMer, *J. Chem. Phys.*, 6, 299 (1938).

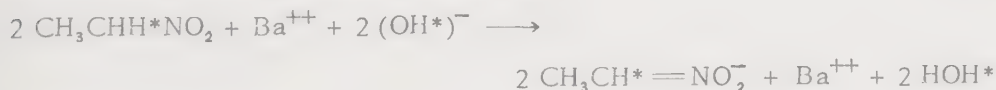
A. Procedure

An equivalent quantity of barium hydroxide- H_2^2 is added to about 0.02 *N* nitroethane in water- H_2^2 to obtain the ion $CH_3CH=NO_2^-$. To the solution is then added an equivalent amount of sulfuric acid- H_2^2 (Note 1). Barium sulfate is precipitated, and a proton- H^2 is introduced into the nitroethane molecule (Note 2).

B. Notes

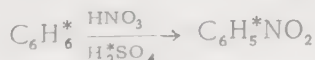
1. Throughout these operations the solution remains perfectly colorless.

2. This is the first recorded instance of the substitution of hydrogen- H^2 for a hydrogen atom changing the color of a compound. If the equivalent quantity of barium hydroxide is again added, the solution turns light yellow very rapidly, and the reaction is:



The color can be discharged and brought back by repeated alternate additions of $H^*_2SO_4$ and $Ba(OH^*)_2$. Maron and LaMer concluded that, in both water and water- H_2^2 , nitroethane, nitroethane-1- H_1^2 , nitroethane-2,2- H_2^2 and the ion $CH_3CH=NO_2^-$ are colorless, while the ion $CH_3CH^*=NO_2^-$ is light yellow in solutions about 0.02 *N* with respect to nitroethane.

NITROBENZENE- H_5^2



T. G. Bonner, F. Bowyer and G. Williams, *J. Chem. Soc.*, 1953, 2650.

A. Procedure (Note 1)

A nitrating acid of the following composition is prepared: sulfuric acid- H_2^{25} , 18.4 g.; water, 3.14 g.; anhydrous nitric acid, 5.29 g. (Note 2). Through a capillary tube, 5.7 g. of benzene- H_2^2 is introduced into the acid mixture, which is maintained at 45° and shaken. The reaction mixture, still maintained at 45° , is shaken for an additional 230 minutes. The temperature of the reaction mixture is raised to 60° at the end of the reaction period. The mixture is poured into water, and the nitrobenzene- H_2^2 is separated and washed with sodium carbonate solution and then with water. The product is dried over phosphorus pentoxide and is then distilled; the yield is 7.7 g. (88.9%). After two distillations a faintly yellow middle fraction of 5.5 g. has the following properties: b.p. $64-65^\circ$ (5 mm.), $215-217^\circ$ (711 mm.); d_4^{25} 1.2533; n_D^{20} 1.5504 (Note 3).

B. Notes

1. Trial experiments indicated the nitration procedure of Mason¹ to give better yields of nitrobenzene, although a slightly more aqueous nitrating acid was found to be advantageous in excluding dinitration.

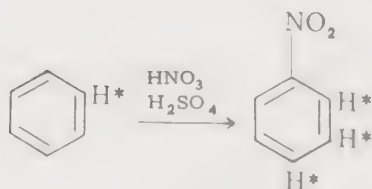
2. To minimize the small risk of isotope exchange during nitration, the nitrating acid was made up with sulfuric acid- H_2^{25} . A sample of nitrobenzene- H_2^2 showed no appreciable exchange when dissolved in 97.3% sulfuric acid at 20° for 1.75 hours.

3. Within the limits of experimental error, the rates of nitration of nitrobenzene and nitrobenzene- H_2^2 were identical in both 97.4% and 86.7% sulfuric acid media. These results are in accord with Melander's² demonstration that scission of the C-H bond is not part of the rate-determining step in aromatic nitrations. The nitration products were not isolated; rather the rate of nitration was followed by measuring the residual nitric acid concentration at known time intervals.³

¹I. Masson, *Nature*, 128, 726 (1931).

²L. Melander, *Arkiv Kemi*, 2, 211 (1950).

³T. G. Bonner, F. Bowyer and G. Williams, *J. Chem. Soc.*, 1952, 3274.

1-NITROBENZENE-2,3,4- $H_{1/3}^2$ 

A. Procedure

Benzene- H_1^2 (91.5 mole %), 20.29 g. (0.256 mole), is nitrated with a mixture of 26.6 ml. (0.42 mole) of nitric acid and 30.4 ml. (0.54 mole) of sulfuric acid according to directions by Fieser,¹ except that the benzene- H_1^2 is added dropwise to the cold nitrating mixture. To complete the reaction, the mixture is heated in a water-bath at 60° for 0.5 hour. After cooling, the two layers are separated, the lower acid layer is discarded, and the organic layer is washed successively with water, dilute sodium hydroxide solution and water. The crude product, 28.7 g., is dried over calcium chloride and distilled (Note 1). A forerun of 0.78 g. (2.5%), b.p. 200–205°, is discarded. The main portion of 19.53 g. (62.2%) is 1-nitrobenzene-2,3,4- $H_1^2/3$, b.p. 205–208.5°, n_D^{20} 1.5528, n_D^{25} 1.5511 (Note 2). An afterrun of 3.13 g. (10.0%), b.p. 210–265°, n_D^{25} 1.5583, is also discarded.

Upon crystallization from ethanol-water the distillation residue yields 3.39 g. (7.8%) of crude 1,3-dinitrobenzene-2,4,5- $H_1^2/3$, m.p. 86–90°, which upon recrystallization yields 1.90 g. (4.4%) of pure product, m.p. 91–92° (Note 3).

B. Notes

1. A Claisen adaptor containing a short length of Cannon protruded packing was used.

2. The nitration of ordinary benzene with nitric acid and sulfuric acid- H_2^2 gave nitrobenzene with no more than the normal deuterium content; hence no exchange occurred under the conditions of nitration. Because of the normal hydrogen isotope effect, exchange of deuterium onto an aromatic nucleus would be expected to occur more readily than its replacement by hydrogen. In another experiment, benzene- H_1^2 was treated with concentrated sulfuric acid under the nitration conditions (50–60° for less than 1 hour); 16.2% of the deuterium was exchanged by hydrogen from the acid, which is considerably less than random distribution of the deuterium. The reverse of this reaction, the deuteration of normal benzene with sulfuric acid- H_2^2 , has been studied by Ingold, Raisin and Wilson,² who found that exchange was independent of sulfonation and could be made to occur even at room temperature, if given sufficient time. They also found³ it impossible to cause deuteration to precede nitration of normal benzene with nitric acid- H^2 . This result was confirmed by Melander⁴ who found that no tritium was introduced into the aromatic nucleus during the dinitration of normal toluene with nitric and sulfuric acids containing a small amount of water- H_2^3 . It was also shown by Lauer and Noland that 1-nitrobenzene-2,3,4- $H_1^2/3$ does not exchange at all with sulfuric acid under the nitration conditions.

3. The nitrobenzene contained 74.0 mole per cent of 1-nitrobenzene-2,3,4- $H_1^2/3$, and the dinitrobenzene contained 60.9 mole per cent of 1,3-

dinitrobenzene-2,4,5- $H_1^2/3$. These are the concentrations expected if there is no isotope effect, which is in agreement with the conclusions of Ingold, *et al.*,⁵ that proton loss during aromatic nitration is kinetically insignificant; hence, the extent of substitution of the nitro group for protium, deuterium or tritium must be directly proportional to the respective concentrations of these isotopes.

¹L. F. Fieser, *Experiments in Organic Chemistry*, 2nd. ed., Heath and Co., Boston, 1941, p. 145.

²C. K. Ingold, C. G. Raisin and C. L. Wilson, *Nature*, 134, 734 (1934); *J. Chem. Soc.*, 1936, 915.

³C. K. Ingold, C. G. Raisin and C. L. Wilson, *ibid.*, 1936, 1637.

⁴L. Melander, *Nature*, 163, 599 (1949); *Arkiv Kemi*, 2, 213 (1950).

⁵R. J. Gillispie, E. D. Hughes, C. K. Ingold, D. J. Millen and R. I. Reed, *Nature*, 163, 599 (1949); E. D. Hughes, C. K. Ingold and R. I. Reed, *J. Chem. Soc.*, 1950 2400.

KETENE- H_2^2



H. R. Johnson and M. W. P. Strandberg, *J. Chem. Phys.* 20, 687 (1952).

A. Procedure

Ketene is usually prepared by the pyrolysis of acetone using a lamp with a Chromel A filament described by Williams and Hurd.¹ Their method of preparation is modified in that a vacuum system (Note 1) is used to minimize loss of the ketene- H_2^2 . 2-Propanone- H_6^2 vapors are passed over the hot filament of the lamp at 700–750°. Most of the 2-propanone- H_6^2 is returned by water-cooled condensers to the heating flask. Ketene- H_2^2 , some 2-propanone- H_6^2 and other products pass through a capillary tube (1 meter long, 0.4 mm. I.D.) into large cold traps immersed in liquid nitrogen (Note 2). Purification of the ketene- H_2^2 is carried out by fractional distillation. The product is first triple-distilled at the temperature of Dry Ice-acetone; this removes impurities less volatile than ketene, mainly 2-propanone- H_6^2 . The ketene- H_2^2 is then placed in a microwave spectroscope and slowly warmed. The absorption of the effluent vapor at the frequency of the $J = 0 \longrightarrow 1$ line is monitored as the vapor is pumped off. This results in discarding impurities more volatile than ketene, from 5 to 20% of the mixture (Note 3).

B. Notes

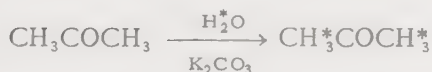
1. A photograph and description of the apparatus are given by Johnson and Strandberg.

2. The pressure on the trap side of the capillary is so low that the traps are essentially 100% efficient in condensing ketene and less volatile products. The pump removes some of a more volatile product, probably methane.

3. Ketene has been stored *in vacuo* for months at liquid nitrogen temperature with no deterioration; some decomposition is apparent in a week of storage at Dry Ice-acetone temperature.

¹J. W. Williams and C. D. Hurd, *J. Org. Chem.*, **5**, 122 (1950).

2-PROPANONE-H₆² (Acetone-H₆²)



F. E. Condon, *J. Am. Chem. Soc.*, **73**, 4675 (1951).

A. Procedure

Acetone-H₆² is prepared from 0.66 mole of acetone by 21 successive exchanges with about 0.6 mole of 99.8% water-H₂² in the presence of about 0.1 g. of anhydrous potassium carbonate. Each exchange is carried out in a 100-ml. flask attached to a fractionating column (Note 1). The acetone-H₂² and a small amount of water-H₂² are distilled through the column to a distillate temperature of about 95° and collected in another 100-ml. flask, which serves as the reaction flask for the next exchange. The final product, obtained in 78% yield, has a deuterium content of 99.3% (H²/H² + H); *n*_D²⁰ 1.3559; *d*²⁰ 0.8749; M.R. 16.00 (Note 2).

B. Notes

1. A 30 × 0.7-cm. column with Heligrid packing was used.
2. The mass spectrum of the final product indicated the presence of 4.1% 2-propanone-H₅².

C. Other Preparations

2-Propanone-H₆² has been prepared¹ by six equilibrations with water-H₂² with sodium hydroxide as catalyst; by a single equilibration with an equal weight of water-H₂², in the presence of potassium carbonate;^{2,3} and by ten equilibrations with water-H₂² containing potassium carbonate.⁴ A study of the reaction kinetics for exchange with water-H₂² has been made by Halford.³ Dadieu and Engler⁵ have prepared 2-propanone-H₆² from barium acetate-H₃².

Leitch⁶ has prepared 2-propanone-H₂⁶ (93.2 mole %) containing small amounts of 2-propanone-H₃² and H₄²-2-propanone by the reaction of acetylene-H₂² with water-H₂² vapor over a catalyst of ferric oxide-zinc oxide at 410°. The distilled product was further enriched by exchange at 60° with water-H₂² containing sodium carbonate.

¹V. J. Shiner, Jr., J. Am. Chem. Soc., 74, 5285 (1952).

²E. W. R. Steacie and W. R. Alexander, Can. J. Research, 15B, 295 (1937).

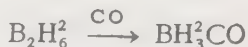
³J. O. Halford, L. C. Anderson, J. R. Bates and R. D. Swisher, J. Am. Chem. Soc., 57, 1663 (1935).

⁴J. R. McNesby, T. W. Davis and A. S. Gordon, *ibid.*, 76, 823 (1954).

⁵A. Dadieu and W. Engler, Naturwiss., 24, 318 (1936).

⁶L. C. Leitch, see T. G. Majury and E. W. R. Steacie, Can. J. Research, 30B, 800 (1952).

BORINE-H₃² CARBONYL



A. B. Burg, J. Am. Chem. Soc., 74, 1340 (1952).

A. Procedure

A mixture of diborane-H₂² (boron hydride-H₂²) at 1 atmosphere and carbon monoxide at 10 atmospheres pressure, in a bomb tube, is reacted at room temperature for 1 week. The borine-H₃² carbonyl is purified by fractional condensation at -150° (Note 1); m.p. -134.5° (Note 2).

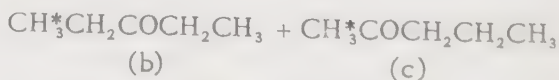
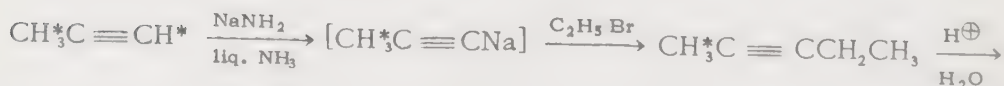
B. Notes

1. The vapor pressures of borine-H₃² carbonyl at -111.85°, -95.5°, -95.4°, and -78.5° are, respectively, 23 mm., 90.6 mm., 91.4 mm. and 294 mm.

2. The melting point of borine-H₃² carbonyl is 2.6° above that of borine carbonyl.¹

¹A. B. Burg and H. I. Schlesinger, J. Am. Chem. Soc., 59, 782 (1937).

3-PENTANONE-1-H₃²



L. C. Leitch and A. T. Morse, Can. J. Chem., 31, 785 (1953).

A. Procedure

(a) *2-Pentyne-1-H₃²*. Sodium amide is prepared by adding 5.7 g. of sodium to 100 ml. of liquid ammonia in a 300-ml. flask equipped with a stirrer, addition tube and a Dry Ice-acetone reflux condenser. Propyne-H₂², 5 l., is distilled into the stirred suspension of sodium amide. Then, 30 g. of ethyl bromide is added, as rapidly as possible, to the sodium methylacetylide-H₃², and the reaction mixture is stirred for 6 hours.¹ Finally, 200 ml. of water is added dropwise, followed by 20 ml. of toluene. The toluene layer is separated, washed with dilute hydrochloric acid and water, dried and fractionated (Note 1). The 2-pentyne-1-H₃² is collected between 54 and 56°; yield 91%.

(b) *3-Pentanone-1-H₃²*. 2-Pentyne-1-H₃², 13.0 g., is stirred at 0° with 69 ml. of 80% sulfuric acid for 2 hours, and the reaction mixture is poured into ice-water.² The yield of mixed ketones, analyzing 37.4% 2-pentanone-1-H₃² and 62.6% 3-pentanone-1-H₃² (Note 2), is 18.5 ml. (82%). The mixed ketones are distilled (vacuum line) into a suspension of 16 g. of sodium bisulfite in 24 ml. of water, and the mixture is shaken for several minutes (Note 3). The unreacted ketone is distilled off and collected in a trap cooled with Dry Ice-acetone. After the treatment with bisulfite is repeated 5 more times, the volume of unreacted ketone is about 8 ml. The product is then distilled through a tube containing pellets of sodium hydroxide to remove sulfur dioxide, and gives only a trace of iodoform on standing in sodium hypiodite solution (Note 4).

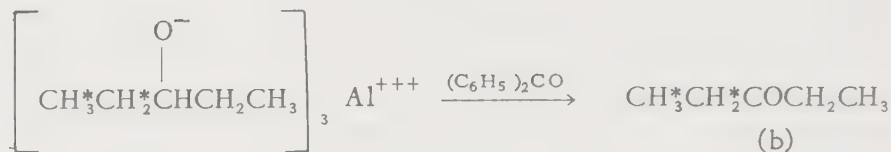
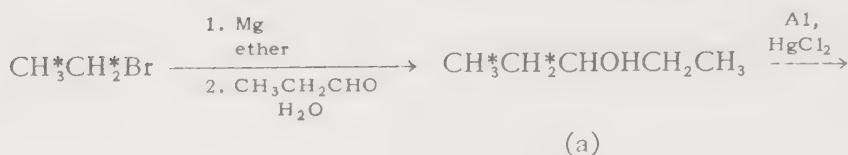
B. Notes

1. A short Stedman column (0.375 × 18 inches) was used.
2. The mixture of ketones was analyzed by mass spectrometry.
3. The separation of the 2-pentanone and 3-pentanone is based upon the procedure of Huijser and Schaafsma,³ who used a continuous counter-current extraction process.
4. This test indicates the absence of a methyl ketone which is, in this case, the more reactive to bisulfite. The product analyzed as follows: C₅H₁₀H₂², 3.17 mole%; C₅H₉H₂O, 7.67 mole%; C₅H₈H₂O, 4.92 mole%; C₅H₇H₃O, 82.2 mole%; C₅H₆H₄O, 0.21 mole%.

¹A. L. Henne and K. W. Greenlee, J. Am. Chem. Soc., 65, 2020 (1943).

²E. L. R. Mowat and J. C. Smith, J. Chem. Soc., 1938, 19.

³H. W. Huijser and A. Schaafsma, U. S. 2,288,281, June 30; through Chem. Abstracts, 37, 139 (1943).

3-PENTANONE-1,2-H₅²

L. C. Leitch and A. T. Morse, Can. J. Chem., 31, 785 (1953).

A. Procedure

(a) *3-Pentanol-1,2-H₅²*. To a solution of ethylmagnesium-H₅² bromide at -5°, prepared from 10.0 ml. of bromoethane-H₅² and 3.0 g. of magnesium (Note 1), is added 10.0 ml. of freshly distilled propionaldehyde. The ether solution is poured onto ice, and the basic magnesium halide is dissolved by the addition of 15% sulfuric acid. The ether solution is separated, and the aqueous layer is extracted with 4 portions of ether which are added to the ether layer. The ether solution is dried over potassium carbonate, filtered and fractionally distilled. The yield of 3-pentanol-1,2-H₅² is 10.2 ml. (71%), b.p. 111-113°.

(b) *3-Pentanone-1,2-H₅²*. A mixture of 10.2 ml. of 3-pentanol-1,2-H₅², 0.81 g. of aluminum shot and a trace of mercuric chloride is heated with a low flame, under reflux, until hydrogen evolution ceases. Unreacted pentanol is then removed under reduced pressure. With stirring, 25 g. of molten benzophenone is added to the residue of aluminum alkoxide. The evolved 3-pentanone-1,2-H₅² is condensed in a trap cooled with Dry Ice-acetone. The yield is 6.7 ml. (75%) (Note 2).

(c) *3-Pentanone-1,2,4-H₇²*. 3-Pentanone-1,2-H₅² is treated three times with water-H₂² containing a few mg. of anhydrous sodium carbonate. This labeled ketone is also prepared by treating 2 ml. of 3-pentanol-1-H₃², in the same manner, with 10-ml. portions of alkaline water-H₂² (Note 3).

(d) *3-Pentanone-2,4-H₄²*. 3-Pentanone, 10 ml., is exchanged 10 times with 10-ml. portions of alkaline water-H₂² (Note 4).

B. Notes

1. See 3-pentanone-H₁₀².
2. Mass spectrometric analysis of this product indicated the following composition: C₅H₄H₆O, 3.72 mole per cent; C₅H₅H₅O, 86.5 mole per

cent; $C_5H_6H_4^2O$, 6.45 mole per cent; $C_5H_7H_3^2O$, 3.49 mole per cent. The atom per cent H^2 was 49.0; n_D^{20} 1.3903.

3. The product, in the former case, contained 87.5 mole per cent $C_5H_3H_7^2O$ and, in the latter, 85.4 mole per cent with varying amounts of the H_3^2 , H_4^2 , H_5^2 , H_6^2 and H_7^2 analogs.

4. The product contained 92.5 mole per cent of $C_5H_6H_4^2O$, and the deuterium content reached a maximum during the exchanges. The presence of 2.78 mole per cent of $C_5H_5H_5^2O$ indicates some exchange in the terminal methyl groups, in addition to 3.7 mole per cent of $C_5H_7H_3^2O$ and 0.78 mole per cent of $C_5H_8H_2^2O$.

C. Other Preparations

3-Pentanone-2,4- H_4^2 has been prepared^{1,2} by heating 3-pentanone under reflux with water- H_2^2 containing potassium carbonate. The exchange reaction was repeated 10 times with fresh samples of water- H_2^2 . Mass spectrometric analysis indicated 98–99% 3-pentanone-2,4- H_4^2 in the product; the remainder was 3-pentanone-2,4- H_3^2 .

¹J. R. McNesby, C. M. Drew and A. S. Gordon, J. Phys. Chem., 59, 988 (1955).

²M. H. J. Wijnen and E. W. R. Steacie, Can. J. Chem., 29, 1092 (1951).

3-PENTANONE- H_{10}^2



L. C. Leitch and A. T. Morse, Can. J. Chem., 31, 785 (1953).

A. Procedure (Note 1)

(a) 3-Pentanone- H_{10}^2 . A solution of 15 ml. of bromoethane- H_2^2 in 25 ml. of ether is added slowly, with stirring, to 5 g. of magnesium turnings in 50 ml. of absolute ether. Finally, the mixture is heated under reflux for 45 minutes, cooled to -20° under an atmosphere of dry argon, and treated with dry carbon dioxide during 1 hour. The reaction mixture is warmed to room temperature under an atmosphere of argon. After removal of ether, the residue is gradually heated to 200° and kept at that temperature for 1 hour while argon is passed through the apparatus. When the residue has cooled to room temperature, it is rapidly transferred (Note 2) to a furnace tube 2 cm. \times 80 cm. (Note 3) and slowly heated to 250° under reduced pressure to remove traces of ether. With the receiver cooled in a bath of Dry Ice-acetone, the temperature of the reactor is slowly raised to 380° and kept there for 1 hour. The apparatus is then filled with argon

and cooled to room temperature. The crude 3-pentanone- H_{10}^2 is shaken with 10 ml. of water- H_2^2 and distilled, on a vacuum line, through Drierite into a receiver cooled in Dry Ice-acetone. The yield is 6.9 g. (74%), n_D^{20} 1.3890 (Note 4).

(b) 3-Pentanone-1,5- H_6^2 . 3-Pentanone- H_{10}^2 is treated with 3 portions of water containing a trace of sodium carbonate (Note 5).

B. Notes

1. In this synthesis, the isolation of deuterated propionic acid is avoided, making unnecessary either the subsequent preparation of the barium salt or the replacement of hydrogen by hydrogen- H^2 in the carboxyl group before pyrolysis.

2. Use of a dry box is preferable.

3. In a reactor, as specified by Ivanoff,¹ the yields of 3-pentanone are consistently 70% or better.

4. The product, which analyzed 70.4 mole per cent $\text{C}_5\text{H}_{10}^2\text{O}$ (94.9 atom per cent H^2), was further enriched in deuterium by exchange at 80° with an equal volume of water- H_2^2 , containing 10 mg. of anhydrous sodium carbonate, in a tube with a break-off seal. The transfer of water- H_2^2 and 3-pentanone- H_{10}^2 before and after each exchange was made in a vacuum manifold. After 4 exchanges, the 3-pentanone- H_{10}^2 analyzed as follows: $\text{C}_5\text{H}_{10}^2\text{O}$, 85.8 mole per cent; $\text{C}_5\text{HH}_9^2\text{O}$, 8.8 mole per cent; $\text{C}_5\text{H}_2\text{H}_8^2\text{O}$, 3.64 mole per cent; $\text{C}_5\text{H}_3\text{H}_7^2\text{O}$, 0.8 mole per cent. This corresponds to a total deuterium content of 97.2 atom per cent. The residual hydrogen in the methyl groups is not exchangeable under the conditions used.

5. Mass spectrometric analysis indicated the following composition: $\text{C}_5\text{H}_4\text{H}_6^2\text{O}$, 88.7 mole per cent; $\text{C}_5\text{H}_5\text{H}_5^2\text{O}$, 8.25 mole per cent; $\text{C}_5\text{H}_6\text{H}_4^2\text{O}$, 2.61 mole per cent; $\text{C}_5\text{H}_3\text{H}_7^2\text{O}$, 0.73 mole per cent.

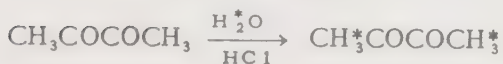
C. Other Preparations

An alternative synthesis of 3-pentanone- H_{10}^2 has been used by Leitch and Morse; 3-pentanol-1,2,4,5- H_{10}^2 is oxidized to the ketone in a neutral, anhydrous medium using a modified Oppenauer oxidation.²

¹M. D. Ivanoff, Bull. soc. chim. France, (4) 43, 441 (1928).

²A. Lauchenauer and H. Schinz, Helv. Chim. Acta, 32, 1265 (1949).

2,3-BUTANEDIONE- H_6^2
(Biacetyl- H_6^2)



D. S. Herr, M. S. Matheson and W. D. Walters, J. Am. Chem. Soc., 63, 1464 (1941).

A. Procedure

A solution of dry, purified biacetyl, about 9%, is prepared in a 1 *N* solution of hydrochloric acid- H^2 in water- H_2^2 . After the solution is kept for 3 days at 56° (Note 1), the biacetyl- H_6^2 is separated in an apparatus similar to that described by Reitz.¹ The major portion of the mineral acid is neutralized with calcium carbonate. Then air is passed, in series, through the biacetyl solution an anhydrous calcium chloride drying train and a trap cooled with Dry Ice. Approximately 85% of the starting material is recovered in the trap (Note 2) and subjected to further exchange in the same manner. The product is distilled several times under high vacuum, and is then dried over anhydrous copper sulfate (Note 3).

B. Notes

1. Walters² has made a kinetic study of the exchange reaction. The rate constant of exchange at 55.8° was 7.5×10^{-4} min.⁻¹.
2. Control experiments with biacetyl indicated that not more than 1.5% was destroyed during the exchange process.
3. It was shown that contact with copper sulfate does not cause deterioration of biacetyl.

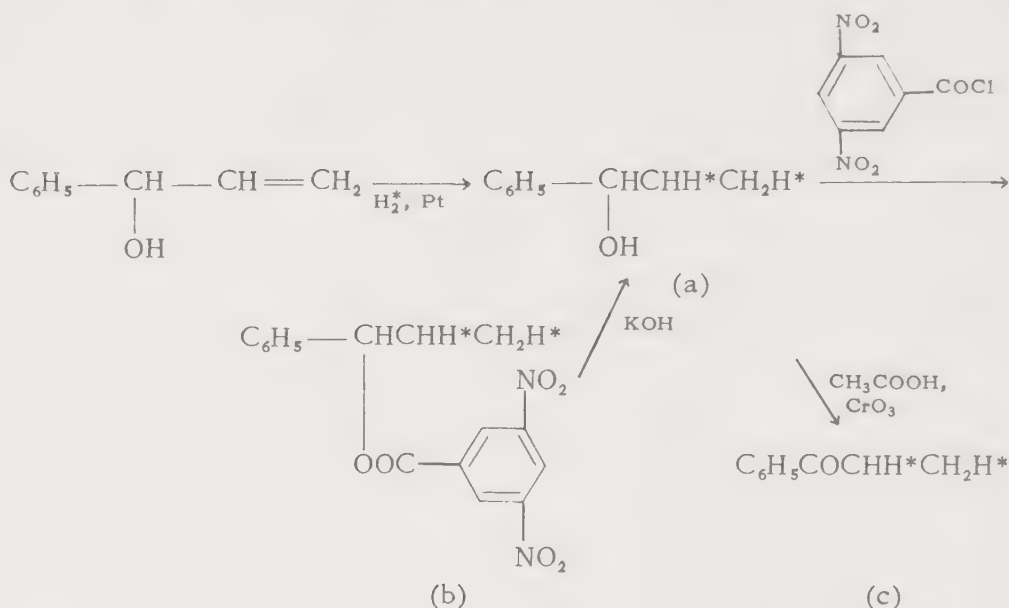
C. Other Preparations

Biacetyl- H_6^2 has been prepared^{2,3} in a manner similar to that described. A very extensive study of the electronic and vibrational states of biacetyl and biacetyl- H_6^2 has been made by the latter authors.

¹O. Reitz, Z. physik. Chem., A179, 119 (1937).

²W. D. Walters, J. Am. Chem. Soc., 63, 2850 (1941).

³J. W. Sidman and D. S. McClure, *ibid.*, 77, 6461 (1955).

PROPIOPHENONE-2,3- H_2^2 

J. B. M. Coppock, J. Kenyon and S. M. Partridge, J. Chem. Soc., 1938, 1069.

A. Procedure

(a) *α-Ethyl-1,2- H_2^2 -benzyl Alcohol*. (–)-*α*-Vinylbenzyl alcohol¹ 15 g. (b.p. 107° at 16 mm.), is hydrogenated with hydrogen- H_2^2 at 2 atmospheres in the presence of platinum oxide catalyst.² Isolated by distillation, the product boils at 207°.

(b) *α-Ethyl-1,2- H_2^2 -benzyl 3,5-Dinitrobenzoate*. *α*-Ethyl-1,2- H_2^2 benzyl alcohol, 15 g., dissolved in 15 g. of pyridine, is added in small portions to 25.5 g. of 3,5-dinitrobenzoyl chloride; the temperature of the mixture is kept below 75°. The pasty mass is warmed at 75° for 30 minutes, then treated with cold, dilute sodium carbonate solution followed by dilute hydrochloric acid, and the ester is extracted with ether. The extract is dried and evaporated to dryness; on trituration with a little hexane, the residue, 31 g., solidifies at once. The product is recrystallized from 96% alcohol, m.p. 52–53° (Note 1).

(c) *Propiophenone-2,3- H_2^2* . To the 14.5 g. of purified *α*-ethyl-1,2- H_2^2 -benzyl 3,5-dinitrobenzoate is added a solution of 4 g. of potassium hydroxide in 50 ml. of ethyl alcohol (Note 2). After heating the mixture 30 minutes on a steam-bath (+)-*α*-ethyl-1,2- H_2^2 -benzyl alcohol is recovered by steam distillation. The colorless alcohol (b.p. 207°), 5.6 g., is mixed with 30 ml. of glacial acetic acid and 4 g. of chromic anhydride is added in small portions at such a rate that the temperature of the mixture remains at 75–80°. Then, sodium hydroxide solution is added, in excess,

and the mixture is steam-distilled. The distillate is salted with potassium carbonate and extracted with ether; propiophenone-2,3- H_2^2 , 4.2 g., crystallizes from the extract as colorless plates, m.p. 19.5° , b.p. 208° . From 4.2 g. of the ketone 5.3 g. of semicarbazone derivative, m.p. 175° , is prepared, having $\alpha \pm 0.01^\circ$ in glacial acetic acid ($c, 5.25$; $l = 2$) (Note 3).

B. Notes

1. The dinitrobenzoate ester was fractionally crystallized from 96% alcohol at 45° . The third crop (14.5 g., m.p. $52-53^\circ$) formed large colorless plates and was used for the subsequent reactions in an attempt to prepare an optically active deuterated ketone.

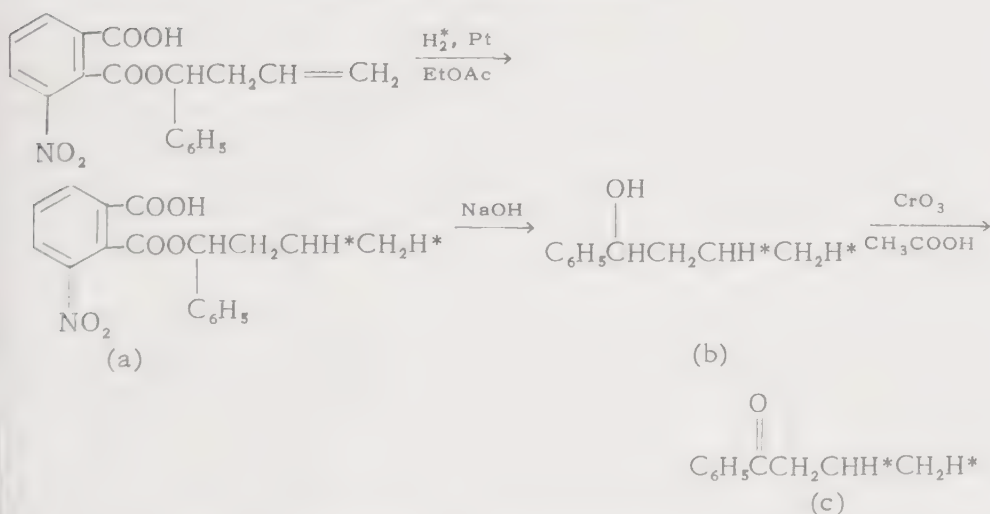
2. The mixture immediately develops an intense violet coloration.

3. The propiophenone-2,3- H_2^2 recovered by hydrolysis of the semicarbazone with hot, dilute sulfuric acid, followed by steam distillation, had zero optical rotation, $\alpha \pm 0.01^\circ$ ($l = 0.25$; $t, 18^\circ$), m.p. 19.5° .

¹D. Duveen, *Compt. rend.*, 206, 1185 (1938).

²*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

BUTYROPHENONE-3,4- H_2^2



A. F. LeC. Holding and W. A. Ross, *J. Chem. Soc.*, 1954, 145.

A. Procedure

(a) 2-(1-Phenylbutoxycarbonyl)-3,4- H_2^2 -3-nitrobenzoic Acid. Hydrogen- H_2^2 is introduced into the evacuated hydrogenation apparatus until the pressure is just below atmospheric. (-)-2-(1-Phenyl-3-butenoxycarbonyl)-3-nitrobenzoic acid, 11.6 g., in 80 ml. of ethyl acetate con-

taining the platinum catalyst, is drawn into the apparatus from a separatory funnel (Note 1). Hydrogenation proceeds smoothly during 2 hours at 40–45°, and the colorless solution becomes a pale yellow. The solution is filtered, and the solvent is removed to obtain 11 g. of the saturated ester, m.p. 128–129°, $[\alpha]_D^{18} -52.6^\circ$ ($l = 4$; c , 5.84 in chloroform), after recrystallization from benzene-petroleum ether. Hydrogenation of an additional 11.6 g. of unsaturated ester, in ether at room temperature, gives 11.6 g. of the same product, m.p. 128–129°, $[\alpha]_D^{18} -53.0^\circ$ ($l = 4$; c , 5.93 in chloroform). After the combined products are recrystallized 14 times from benzene-petroleum ether, the yield of 2-(1-phenylbutoxycarbonyl-3,4- H_2^2)-3-nitrobenzoic acid, m.p. 131–132°, is 6.6 g.; $[\alpha]_D^{20} -54.1^\circ$ ($l = 4$; c , 5.82 in chloroform).

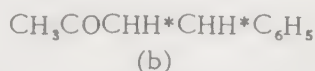
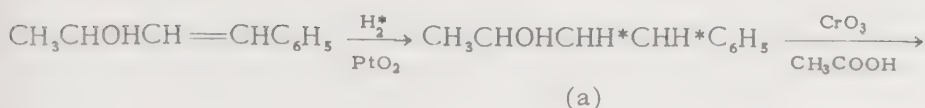
(b) (-)-1-Phenyl-1-butanol-3,4- H_2^2 . The above 6.6 g. of saturated ester is hydrolyzed in an excess of sodium hydroxide, and the resulting alcohol is isolated by steam-distillation. The yield of (-)-1-phenyl-1-butanol-3,4- H_2^2 , which is extracted from the distillate with ether, is 2.7 g.; m.p. 45–46°, $[\alpha]_D^{20} -44.7^\circ$ ($l = 4$; c , 5.17 in benzene).

(c) Butyrophenone-3,4- H_2^2 . To 2.7 g. of 1-phenyl-1-butanol-3,4- H_2^2 , in 15 ml. of acetic acid at 40–45°, is added 1.8 g. of chromic oxide in 17 ml. of 88% aqueous acetic acid. After the mixture is stirred for an additional 30 minutes, the excess of chromic oxide is decomposed with a few drops of sulfurous acid, and the mixture is neutralized with sodium carbonate solution. The product is distilled with steam, and extraction of the distillate with steam gives 2.3 g. of butyrophenone-3,4- H_2^2 , as a pale yellow liquid. Butyrophenone-3,4- H_2^2 semicarbazone, 3.1 g., is prepared in aqueous ethanol; m.p. 188–190°. The free ketone, 2.0 g., is recovered by treating 3.0 g. of the semicarbazone with sulfuric acid and distilling the product with steam; $n_D^{18.5} 1.5162$ (Note 2).

B. Notes

1. The preparation of 2-(1-phenyl-3-butenoxycarbonyl)-3-nitrobenzoic acid and its resolution into the two optical antipodes is described by Holding and Ross.

2. Neither the butyrophenone-3,4- H_2^2 nor its semicarbazone derivative showed any optical activity.

4-PHENYL-2-BUTANONE-3,4-H₂²

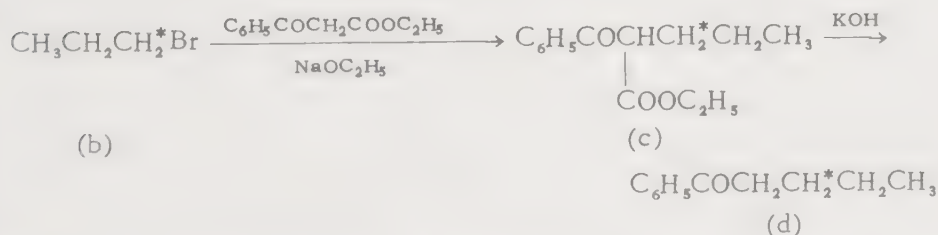
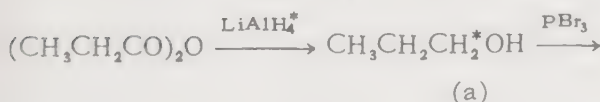
J. B. M. Coppock and S. M. Partridge, *Nature*, 137, 907 (1936).

A. Procedure

(a) *4-Phenyl-2-butanol-3,4-H₂²*. D-4-Phenyl-3-buten-2-ol, in ether solution, is hydrogenated with hydrogen-H₂² in the presence of a platinum oxide catalyst.¹ The product is fractionally distilled, b.p. 127° (18 mm.).

(b) *4-Phenyl-2-butanone-3,4-H₂²*. In acetic acid solution, 4-phenyl-2-butanol-3,4-H₂² is oxidized with chromic acid at 75–80°. The resulting ketone is purified by preparation of the bisulfite addition compound which is decomposed with sodium carbonate solution. The product, b.p. 232°, displays no optical rotation.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley New York, 1941, p. 463.

VALEROPHENONE-3-H₂²

E. Cerwonka, R. C. Anderson and E. V. Brown, *J. Am. Chem. Soc.*, 75, 30 (1953).

A. Procedure

(a) *1-Propanol-1-H₂²* (Note 1). Lithium aluminum hydride-H₄², 8.913 g. (0.212 mole), is finely divided and suspended in 1 l. of anhydrous diethyl carbitol (Note 2). To this suspension is added with stirring, 26.0 g.

(0.20 mole) of propionic anhydride dissolved in an equal volume of anhydrous diethyl carbitol. With the temperature maintained below 50° , stirring is continued for 6 hours, and the mixture is allowed to stand for 1 hour. After the addition of 240 g. of purified monobutyl carbitol, the 1-propanol-1- H_2^2 is distilled off under reduced pressure (Note 3). The distillate is collected in a cold-trap until only the ether is being distilled. Fractionation of the distillate gives 9.9 g. (40%) of 1-propanol-1- H_2^2 , boiling range $96-97.5^{\circ}$.

(b) *1-Bromopropane-1- H_2^2* . This synthesis is an adaptation of the preparation of isobutyl bromide¹ by treatment of the corresponding alcohol with phosphorus tribromide. From 8.6 g. of 1-propanol-1- H_2^2 there is obtained 8.2 g. (47%) of 1-bromopropane-1- H_2^2 .

(c) *Ethyl 2-Benzoylvalerate-3- H_2^2* . The following procedure is a modification of that of Perkin and Calman.² Sodium ethoxide is prepared from 1.6 g. (0.70 mole) of sodium in 80 ml. of absolute ethanol. The solution is cooled to 0° , and 13.73 g. (0.072 mole) of ethyl benzoylacetate is added with shaking. The mixture is kept cold for 1 hour, and then 8.2 g. (0.066 mole) of 1-bromopropane-1- H_2^2 is added, and the mixture is heated under reflux for a period of 4 hours.

After excess alcohol is distilled, the oily residue is cooled, washed with water several times (Note 4) and dried over anhydrous sodium sulfate. The product, distilled under reduced pressure, is collected over a boiling range of $113-119^{\circ}$ (2 mm.). The mixture of ethyl 2-benzoylvalerate-3- H_2^2 and unreacted ethyl benzoylacetate weighs about 13 g. (Note 5).

(d) *Valerophenone-3- H_2^2* . The crude ester is added to a solution of 4.5 g. of potassium hydroxide in 120 ml. of ethanol and 60 ml. of water. This mixture is heated on a steam-bath for 6 hours and, after cooling, the oily ketone-layer is separated, dried and fractionated under reduced pressure. Acetophenone, resulting from decarboxylation of unreacted ethyl benzoylacetate, is distilled at $72-75^{\circ}$. The valerophenone-3- H_2^2 then distills at $104-106^{\circ}$; the yield is 3.4 g. (33%) (Note 6).

B. Notes

1. Since anhydrous 1-propanol was required for preparation of the bromide, water was excluded completely by adapting the synthesis of anhydrous methanol using lithium aluminum hydride.³

2. Active hydrogen compounds may be removed from diethyl carbitol by distilling it first from sodium and then from lithium aluminum hydride. Since both reagents split ethers at temperatures above 60° ,⁴ distillation under vacuum is essential.

3. The butyl carbitol serves both to decompose excess lithium aluminum hydride and to liberate the propanol.

4. To remove sodium bromide and traces of alkali.

slowly to the stirred suspension of aluminum chloride during 2 hours. The mixture is heated under reflux an additional 2 hours, cooled and poured slowly into 45 ml. of concentrated hydrochloric acid and 60 g. of ice. The solid product is collected and distilled. The yield of 1-naphthyl-2-H² ketone, b.p. 207–208° (6 mm.), is 17.6 g. (73%), m.p. 70–71°.

2-HYDROXYETHYLTRIMETHYL-H₃-AMMONIUM CHLORIDE (H₃-Choline Chloride)

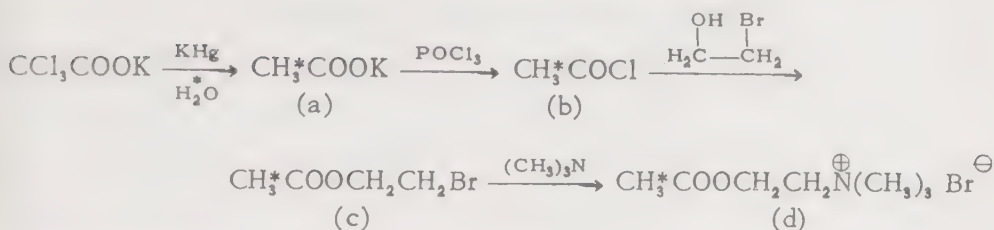


V. du Vigneaud, M. Cohn, J. P. Chandler, J. R. Schenck and S. Simmonds, J. Biol. Chem., 140, 625 (1941).

Procedure

The methyl-H₃² iodide, prepared by the action of red phosphorus and iodine on 4.28 g. of methyl-H₃² alcohol, is distilled through a water-cooled condenser into a flask containing 8.8 g. of ethanolamine and cooled in an ice-salt bath. The mixture is kept at room temperature with occasional shaking for 60 hours. The contents of the flask are then dissolved in 80 ml. of 0.2 N sodium hydroxide, and to the solution is added 550 ml. of a saturated aqueous solution of ammonium reineckate (ammonium salt of Reinecke's acid, tetrathiocyanodiammonochromic acid). The mixture is cooled overnight to effect complete precipitation of H₃²-choline reineckate. After it is collected and dried, the H₃²-choline salt weighs 17.24 g. (64% based on methyl-H₃² alcohol). The salt is decomposed by the method of Kapfhammer and Bischoff.¹ The H₃²-choline reineckate is dissolved in acetone-water (1:1), an equivalent amount of silver sulfate solution (6 g./l.) is added, and the silver reineckate is filtered off. The filtrate is then treated with barium chloride solution until it is completely free of sulfate ions. The clear filtrate is then evaporated to dryness at 35–40° *in vacuo*. The residue is extracted with absolute alcohol and the solution is filtered and again concentrated to dryness. The H₃²-choline chloride weighs 3.45 g.

¹J. Kapfhammer and C. Bischoff, Z. physiol. Chem., 191, 179 (1930).

ACETYL-H₃²-CHOLINE BROMIDE

H. Erlenmeyer and H. Lobeck, *Helv. Chim. Acta*, 20, 142 (1937).

A. Procedure

(a) *Potassium Acetate-H₃²*. Dry potassium trichloroacetate dissolved in water-H₂² is treated with potassium amalgam. The reaction mixture is saturated with carbon dioxide and evaporated to dryness. The product is then extracted from the residue with absolute alcohol. After the solvent is removed, the potassium acetate-H₃² is dried by fusion under vacuum.

(b) *Acetyl-H₃² Chloride*. The dry potassium acetate-H₃² is converted to acetyl-H₃² chloride by treatment with phosphoryl chloride. The acid chloride distills at 47–51°.

(c) *2-Bromoethyl Acetate-H₃²*. A solution of acetyl-H₃² chloride in dry ether is treated with an ether solution of ethylene bromohydrin and one equivalent of pyridine. Water is added; the ether layer is washed with dilute sulfuric acid and with sodium carbonate solution and dried. After removal of ether, the 2-bromoethyl acetate-H₃² is distilled.

(d) *Acetyl-H₃²-choline Bromide*. According to the procedure of Fourneau and Page,¹ 2-bromoethyl acetate-H₃² is treated with a solution of trimethylamine in benzene at 100°. The reaction is complete in four hours, and the resulting acetyl-H₃²-choline bromide is twice recrystallized from absolute ethanol; yield, 87%.

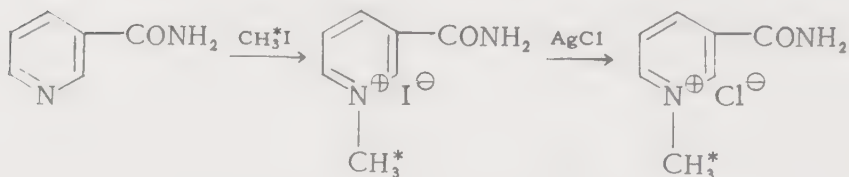
C. Other Preparations

Acetyl-H₃² chloride has been prepared by treating acetic-H₃² acid-H² with phosphorus trichloride.²

¹E. Fourneau and H. J. Page, *Bull. soc. chim. France*, 15, 544 (1914).

²W. Engler, *Z. physik. Chem.*, B35, 433 (1937).

3-CARBAMOYL-1-METHYL-H₃²-PYRIDINIUM CHLORIDE
(N¹-Methyl-H₃²-nicotinamide Chloride)



E. B. Keller, J. L. Wood and V. du Vigneaud, *Proc. Soc. Exptl. Biol. Med.*, 67, 182 (1948).

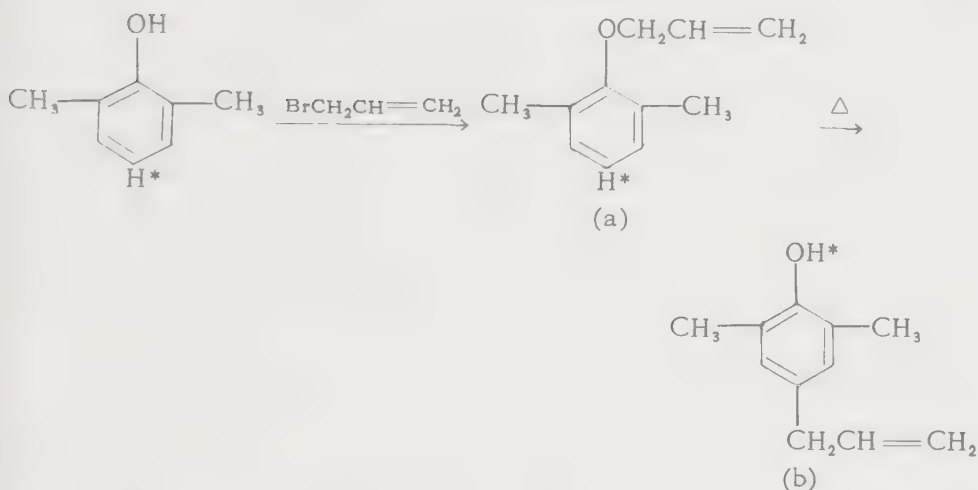
Procedure

(a) *3-Carbamoyl-1-methyl-H₃²-pyridinium Iodide, (N¹-Methyl-H₃²-nicotinamide Iodide)*. This compound is prepared from methyl-H₃² iodide and nicotinamide by adaptation of the following method of Karrer.¹ To 5 g. of finely pulverized nicotinamide is added 30 ml. of methyl iodide, and the suspension is gently refluxed for 6 hours. After the excess methyl iodide is removed by distillation, the yellow-colored residue is dissolved in a minimum of hot water, about 5 ml. The solution is filtered and diluted with 20 ml. of absolute alcohol, and the crystalline, yellow-colored N¹-methylnicotinamide iodide precipitates upon the addition of 100-150 ml. of ether. The yield of product, m.p. 204°, is 8-9 g. This compound is quite soluble in water, difficultly soluble in alcohol, and insoluble in ether.

(b) *3-Carbamoyl-1-methyl-H₃²-pyridinium Chloride, (N¹-Methyl-H₃²-nicotinamide Chloride)*. The above N¹-methyl iodide compound in aqueous solution is shaken with freshly prepared silver chloride. The N¹-methylnicotinamide chloride melts at 240°.

¹P. Karrer, G. Schwarzenbach, F. Benz and U. Solmssen, *Helv. Chim. Acta*, 19, 811 (1936).

4-ALLYL-2,6-XYLENOL-H²
(4-Allyl-2,6-dimethylphenol-H²)



G. B. Kistiakowsky and R. L. Tichenor, J. Am. Chem. Soc., 64, 2302 (1942).

A. Procedure

(a) *Allyl 2,6-Xylyl-4-H² Ether*. To 13.5 g. (0.34 mole) of sodium hydroxide in a 3-necked 250 ml. flask, equipped with a wire stirrer, reflux condenser and dropping funnel, are added quickly, in this order, 25 ml. of water, 15 ml. of freshly distilled allyl bromide, 25 ml. of acetone and 13 g. (0.106 mole) of 2,6-xylenol-4-H². The mixture is heated under reflux for 2 hours with stirring, cooled, and extracted with petroleum ether. The extract is washed with sodium hydroxide, then with water and dried over magnesium sulfate (Note 1). After concentration by distillation on the steam-bath, the allyl 2,6-xylyl-4-H² ether is distilled at 2 mm. through a small Podbielniak column (Note 2).

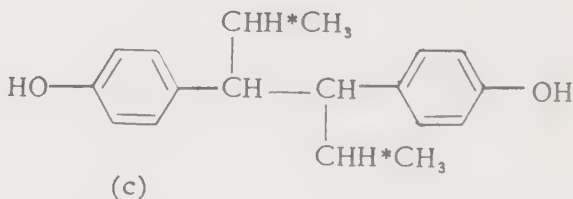
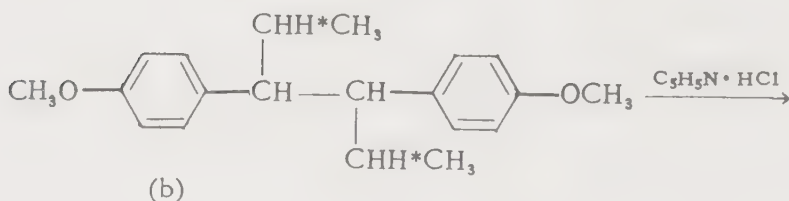
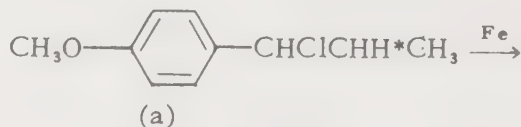
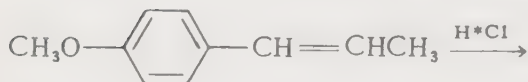
(b) *4-Allyl-2,6-xylenol-H², (4-Allyl-2,6-dimethylphenol-H²)*. The purified allyl 2,6-xylyl-4-H² ether is placed in a clean Pyrex tube, frozen evacuated, melted, refrozen and evacuated to remove dissolved gases. The tube is sealed and heated in a Wood's metal-bath until samples of the same ether without deuterium, heated simultaneously, are found to be almost entirely soluble in 20% sodium hydroxide solution. At a bath temperature of 190–200°, allyl 2,6-xylyl ether is practically all rearranged in 4–5 hours. The purified phenol melts at 26–27°; n_D^{25} 1.5356.

B. Notes

1. A neutral drying agent is used to minimize possible exchange of deuterium.

2. A faint yellow color was present in all the preparations of this ether.

meso-4,4'-[1,2-BIS(ETHYL-1- H_1^2)ETHYLENE]DIPHENOL
(H_2^2 -Hexestrol)



A. Lacassagne, Ng.-Ph. Buu-Hoï, A. Chamorow, N. Dat-Xuong and N. Hoan., Compt. rend., 231, 1384 (1950).

A. Procedure (Note 1)

(a) *p*-(1-Chloropropyl-2- H_2^2)anisole. This compound is prepared in almost quantitative yield by saturating an ice-cooled mixture of α -ethyl-anisyl alcohol and petroleum ether with dry hydrogen- H_2^2 chloride (Note 2). However, the addition of hydrogen chloride to anethole is more convenient. An ice-cooled mixture of 200 g. of crystallized anethole and 40 ml. of petroleum ether is saturated with dry hydrogen- H_2^2 chloride, and the dark-colored liquid obtained is thoroughly washed with ice-water (Note 3).

(b) α, α' -Bis(ethyl-1- H_1^2)-4,4'-dimethoxybibenzyl. The above liquid is added in small portions (10-ml.) to a well-stirred mixture of 80 g. of hydrogen-reduced iron powder and 1000 ml. of water previously heated at 75–80°. A brisk reaction is noted after each addition, and most of the

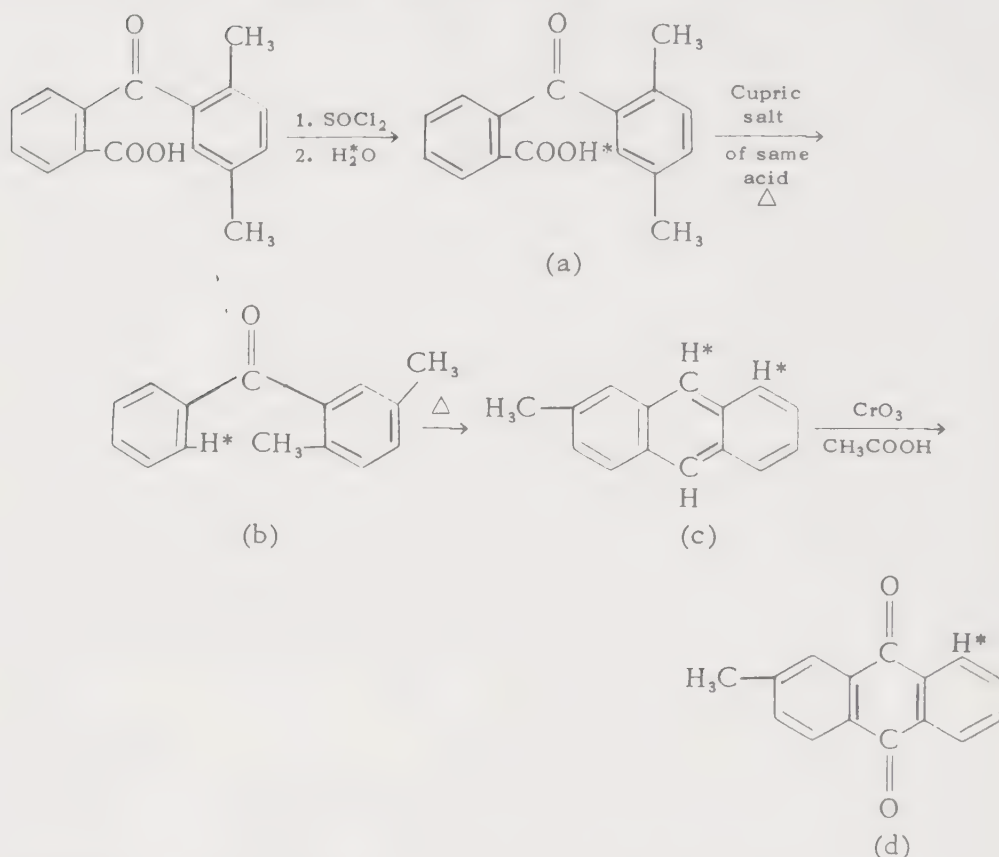
petroleum ether is allowed to distill off. After the addition is complete, the reaction mixture is boiled for 30 minutes and left overnight at 2°. The partially solidified upper organic layer is collected on a filter, and the product is freed of iron powder by extraction into benzene. The filtered benzene solution is evaporated to dryness, and the residue is recrystallized from methanol to obtain 17 g. of α, α' -bis(ethyl-1- H_1^2)-4,4'-dimethoxybibenzyl, m.p. 147-148°. The aqueous filtrate is also extracted with benzene; after removal of the solvent, vacuum-distillation of the remaining oil affords 50 g. of anethole, b.p. 232° (containing some propylanisole, b.p. 212°-218°), and 62 g. of an oil, b.p. 222-235° (13 mm.), which partly solidifies giving a second crop of the product. The remaining oil, dissolved in methanol and kept in a refrigerator for some time, affords a third crop of the product making the total yield 36 g. (Note 4).

(c) *meso*-4,4'-[1,2-Bis(ethyl-1- H_1^2)ethylene]diphenol, (H_2^2 -Hexestrol). A mixture of 300 g. of anhydrous pyridinium chloride and 100 g. of *meso*- α, α' -bis(ethyl-1- H_1^2)-4,4'-dimethoxybibenzyl is gently refluxed until the second layer disappears. Heating is continued for 10 minutes, and the hot reaction mixture is poured into 1 l. of ice-water with stirring. The nearly colorless solid is collected, dried and crystallized from benzene, m.p. 187°. The yield in the demethylation step is 92%.

B. Notes

1. The isotopic synthesis was patterned after the procedure described by Buu-Hoï and Hoan.¹
2. Dry hydrogen- H^2 chloride is prepared by the action of water- H_2^2 on thionyl chloride.
3. This treatment changed the color of the solution to a pale pink.
4. Distillation of the filtrate from the latter operation gave 41 g. of *racemic*- α, α' -bis(ethyl-1- H_1^2)-4,4'-dimethoxybibenzyl, b.p. 225-230° (13 mm.).

¹Ng. Ph. Buu-Hoï and Ng. Hoan, J. Org. Chem., 14, 1023 (1949).

2-METHYLANTHRAQUINONE-8- H^2 

C. D. Hurd and J. Azorlosa, J. Am. Chem. Soc., 73, 37 (1951).

A. Procedure

(a) *2-(2,5-Dimethylbenzoyl)benzoic Acid- H^2* . Nonisotopic 2-(2,5-dimethylbenzoyl)benzoic acid, 47.4 g., is heated under reflux for 2 hours with 80 ml. of redistilled thionyl chloride. Excess thionyl chloride is removed, first by heating at 60° under reduced pressure, and then by twice adding 30-40 ml. of dry toluene which is distilled off under vacuum with a steam-bath. The cream-colored residue is dissolved in 150 ml. of dry toluene, and 4 ml. of water- H^2 (99.5%) is added. The mixture is stirred and heated on a steam-bath for 1 hour. The evolved hydrogen- H^2 chloride is dissolved in sodium hydroxide solution. The 2-(2,5-dimethylbenzoyl)benzoic acid- H^2 , which separates upon cooling, is collected and recrystallized from ligroin-toluene (1:1) (Note 1). Additional material is obtained by concentrating the filtrate, and, in all, 41.6 g. (88%) of the acid- H^2 is obtained.

(b) *2,5-Dimethylbenzophenone-2'- H^2* . 2-(2,5-Dimethylbenzoyl)benzoic acid- H^2 , 40 g., is placed in a 125-ml. distilling flask connected in series

to a trap, a gas bubbler containing a little sulfuric acid and an aspirator pump. Any moisture is expelled by heating the acid- H^2 to 200° and flaming the walls of the apparatus. Then, 1.7 g. of the dry cupric salt of the same acid is added, the mixture is heated rapidly to $260-270^\circ$, and this temperature is maintained for 1 hour (Note 2). When carbon dioxide evolution ceases, the mixture is cooled, and the product is dissolved in 50 ml. of ether. This solution is washed twice with 20-ml. portions of 10% sodium hydroxide solution, then with water, and dried over calcium chloride. After removal of ether, distillation of the product yields 22.1 g. of 2,5-dimethylbenzophenone-2'- H^2 , b.p. $126-129^\circ$ (2 mm.) (Note 3).

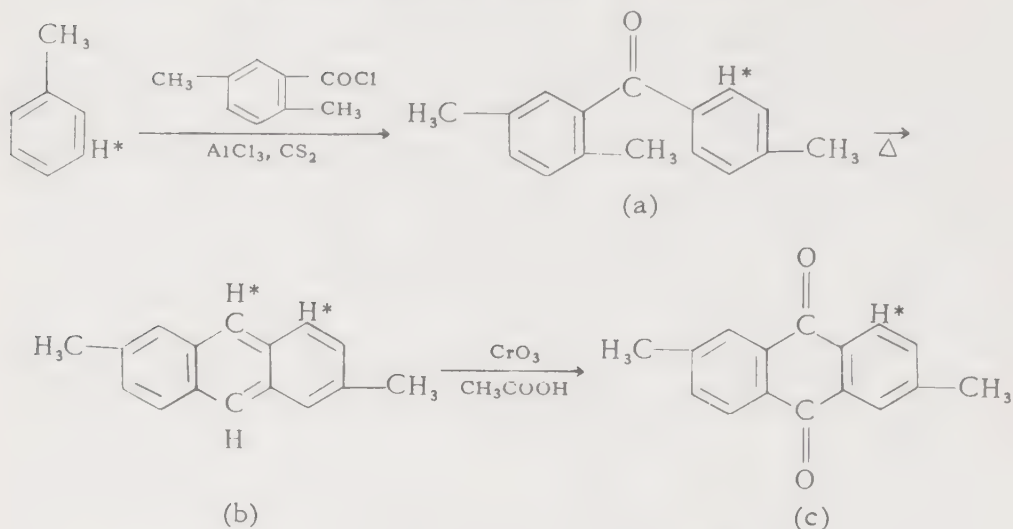
(c) 2-Methylanthracene-8,9- $H^2_{1/2}$. The 2,5-dimethylbenzophenone-2'- H^2 , 20.0 g., is heated at $325-334^\circ$ for 48 hours, and then the unreacted ketone, extracted with ether, is heated an additional 15 hours (Note 4). The total crude product, 7.8 g., is recrystallized from carbon disulfide and benzene to obtain 2.1 g. of 2-methylanthracene-8,9- $H^2_{1/2}$, m.p. $204-205^\circ$.

(d) 2-Methylanthraquinone-8- H^2 . To 3 g. of 2-methylanthracene-8,9- $H^2_{1/2}$, dissolved in about 20 ml. of hot glacial acetic acid, is added slowly a solution of 4.8 g. of chromic anhydride in 8 ml. of water. After the mixture is heated under reflux for 30 minutes, it is poured into 50 ml. of water. The insoluble 2-methylanthraquinone-8- H^2 is recrystallized from ethanol; yield 1.9 g., m.p. $172-174^\circ$ (Note 5).

B. Notes

1. The product is protected with a drying tube during this operation.
2. Daugherty's general procedure¹ is used.
3. This product, a light yellow liquid, did not solidify upon standing.
4. See the description of apparatus and procedure given for 2,6-dimethylanthracene-8,9- $H^2_{1/2}$.
5. These compounds were used in a study of the mechanism of the Elbs reaction; also see 2,6-dimethylanthraquinone-8- H^2 , Note 6. The relative deuterium contents of compounds (b), (c) and (d) were, respectively, 1.000, 0.790 and 0.613.

¹G. Daugherty, J. Am. Chem. Soc., 50, 571 (1928).

2,6-DIMETHYLANTHRAQUINONE-8- H^2 

C. D. Hurd and J. Azorlosa, J. Am. Chem. Soc., 73, 37 (1951).

A. Procedure

(a) *2,4',5-Trimethylbenzophenone-2'-H²*. In a 3-necked, 500-ml. flask, equipped with stirrer, dropping funnel and reflux condenser, are placed 27 g. of anhydrous aluminum chloride and 60 ml. of carbon disulfide. Over a period of 2 hours, a solution of 27.1 g. of 2,5-dimethylbenzoyl chloride and 15 g. of toluene-3- H^2 in 40 ml. of carbon disulfide is slowly added to the stirred suspension of aluminum chloride. The mixture is then heated under reflux for 2 hours. After cooling, it is poured into 75 ml. of concentrated hydrochloric acid and 100 g. of ice. Then, 20 ml. of ether is added, and the two phases are separated; the water layer is extracted twice with 60-ml. portions of ether. The combined ether and carbon disulfide solution is washed with dilute sodium hydroxide and dried over calcium chloride. Removal of solvent and distillation of the residue yields 28 g. of product, b.p. 157–160° (3 mm.). This material is redistilled to obtain 26.2 g. (72%) of 2,4',5-trimethylbenzophenone-2'- H^2 , b.p. 157–160° (3 mm.) (Note 1).

(b) *2,6-Dimethylanthracene-8,9-H_{1/2}²*. For pyrolysis of the ketone, a dry, 50-ml., 2-necked reaction flask is used which is equipped with a thermometer that dips into the reaction mass, and a column. The latter is maintained at 115–135° by an electrical heating jacket (Note 2). Connected to the column is a small ice-cooled trap with a calcium chloride tube at the exit. The reaction mixture itself is heated by a fused salt-bath of sodium nitrite and sodium nitrate.

2,4',5-Trimethylbenzophenone-2'- H^2 , 16.4 g., is heated at 350° for 8 hours; the unreacted ketone is extracted with ether (Note 3) and again

heated for 5 hours after removal of the ether. The water from the reaction is freed from entrained organic matter by shaking with ether (Note 4). The residue, after heating, is dark red or brown in color if reaction has occurred (Note 5), and the high melting 2,6-dimethylantracene solidifies on cooling. Extraction of the fusion product with ether leaves 5.0 g. of crude 2,6-dimethylantracene-8,9- H_1^2 , which is purified by recrystallization from carbon disulfide and benzene, m.p. 235–238°.

(c) 2,6-Dimethylantraquinone-8- H^2 . 2,6-Dimethylantracene-8,9- H_1^2 (Note 6), 2.0 g., is dissolved in 15 ml. of hot acetic acid, to which is slowly added a solution of 3.0 g. of chromic anhydride in 5 ml. of water. The mixture is heated under reflux for 30 minutes and poured into 50 ml. of water. The insoluble 2,6-dimethylantraquinone-8- H^2 is collected and crystallized from ethanol; m.p. 239–240°.

B. Notes

1. Attempts to crystallize this liquid product were unsuccessful.
2. Thus, condensation of water is prevented, but higher boiling components are retained.
3. The unused ketone, soluble in ether, is extracted from the reaction mass to lessen any thermal reaction with the anthracene formed.
4. Since this work was a study of the mechanism of the Elbs reaction,^{1,2,3} the water formed is collected for deuterium analysis, shaken with ether and distilled after addition of a crystal of potassium permanganate.
5. When heated at 325° for 9 hours this ketone changed very little.
6. On a relative basis, the deuterium contents of the substituted ketone, anthracene, anthraquinone and water formed in pyrolysis of the ketone are respectively: 1.000, 0.676, 0.518 and 0.010. Apparently, the anthracene had deuterium in positions 8 and 9, the ring closure favored the *ortho*-hydrogen over the *ortho*-deuterium, and the hydrogen atom appearing at the 9-position in anthracene comes from the *ortho*-nuclear position and not from the methyl group.

C. Other Preparations

1,4-Bis(hydroxy- H^2)anthraquinone, (H_2^2 -quinizarin), 1,5-bis(hydroxy- H^2)-anthraquinone, (H_2^2 -anthrarufin), and 1,4,5,8-tetrakis(hydroxy- H^2)anthraquinone have been prepared⁴ by bubbling dry carbon dioxide through suspensions of the corresponding potassium salts in water- H_2^2 .

¹L. F. Fieser and E. M. Dietz, *Ber.*, 62, 1827 (1929).

²J. W. Cook, *J. Chem. Soc.*, 1931, 487.

³G. T. Morgan and E. A. Coulson, *ibid.*, 1931, 2323.

⁴D. Hadži and N. Sheppard, *Trans. Faraday Soc.*, 50, 911 (1954).

hours. From the reaction mixture is obtained a yellow oil which is chromatographed on acid-washed alumina to obtain the H^2 -4-cholesten-3-one, m.p. 78.5–80.5° (Note 3).

(b) *H²-Cholesteryl Acetate*. H^2 -Cholesterol is acetylated with acetic anhydride and pyridine at room temperature. After recrystallization from ethanol, the product melts at 114.5–116.5°.

(c) *H²-3 β -Acetoxy-5-androsten-17-one, (H^2 -Dehydroisoandrosterone Acetate)*. To a solution of 12.00 g. of H^2 -cholesteryl acetate in 48.5 ml. of ethylene chloride containing 2.5 ml. of acetic acid is added a solution of 1.55 ml. of bromine and 1.2 ml. of pyridine in 102.5 ml. of ethylene chloride. This mixture is stored in the refrigerator for 2 days. The solution of brominated cholesteryl acetate is then diluted with 21.5 ml. of ethylene chloride and 125 ml. of acetic acid and chilled to 12°. To the cold solution is added, during 9.5 hours, 184 ml. of a solution containing 28 g. of chromic acid in 36 ml. of water, 130 ml. of acetic acid and 23.2 ml. of concentrated sulfuric acid. The temperature of the mixture is maintained between 16–20° during this addition. The mixture then is stirred at 20° for another 13 hours, and excess chromic acid is destroyed with ethanol. After 1 l. of 15% sodium chloride solution is added, the mixture is exhaustively extracted with ether. The combined ether extracts are washed with 10% sodium chloride solution. The ether solution is dried over sodium sulfate, and distillation of the solvent leaves a yellow oil. The crude product is dissolved in 200 ml. of acetic acid, and 10 g. of zinc dust is added portion-wise within a period of 1.5 hours with the temperature of the solution at 20°. After the mixture is stored for 12 hours at room temperature, the organic material is extracted into ether, washed with 10% sodium chloride solution and dried over sodium sulfate. The solvent is evaporated to obtain 12.9 g. of oily, halogen-free product, which is partitioned between ether and 16% sodium hydroxide solution to give 2.70 g. of neutral material and 8.53 g. of acidic material.

The neutral fraction is treated with Girard's Reagent T, and 1.415 g. of ketonic and 1.242 g. of non-ketonic material are obtained. The ketonic fraction is refluxed with 75 ml. of methanol and 50 ml. of 5% methanolic potassium hydroxide for 2 hours under nitrogen. From the hydrolysis mixture is obtained 1.095 g. of reddish oil. The product is chromatographed on acid-washed alumina and eluted with benzene to obtain 565 mg. of crude H^2 -dehydroisoandrosterone. This product is acetylated with acetic anhydride and pyridine at room temperature, and the acetate is recrystallized from ethanol and petroleum ether, m.p. 168–169.5°; $[\alpha]_D^{20} -5.9^\circ$ (chloroform).

(d) *H²-4-Androstene-3,17-dione*. H^2 -Dehydroisoandrosterone acetate, 163 mg., is hydrolyzed by refluxing with methanolic potassium hydroxide for 15 minutes. The hydrolyzed product is oxidized by the Oppenauer method, and the crude H^2 -androstenedione thus obtained is refluxed under nitrogen for 2 hours with 25 ml. of methanol, 15 ml. of 5% methanolic po-

potassium hydroxide and 5 ml. of water. Chromatographic separation on acid-washed alumina and recrystallization from acetone-petroleum ether give H^2 -4-androstene-3,17-dione; m.p. 170.5–171.5°.

(e) *Methyl H^2 -3 β -Acetoxy-5-cholenate*. The acidic fraction, 8.53 g., from the chromic acid oxidation of H^2 -cholesteryl acetate is selectively methylated at room temperature with 400 ml. of absolute methanol and 1 ml. of concentrated sulfuric acid. A portion of the methanol is removed *in vacuo*, and the residual mixture is dissolved in ether. The ether solution is washed: first with water, repeatedly with saturated sodium bicarbonate solution, and again with water. From the ether solution, upon evaporation, is obtained 4.27 g. of an oily mixture of methyl esters. The neutral ester mixture, 4.27 g., is treated with acetic anhydride and pyridine at room temperature overnight to obtain 4.61 g. of amber oil, which is then chromatographed on acid-washed alumina. The benzene-petroleum ether (1:9) eluate yields an oil which on recrystallization from acetone-methanol gives methyl H^2 -3 β -acetoxy-5-cholenate, m.p. 156.5–157.5°; $[\alpha]_D^{28} -43^\circ$ (chloroform).

(f) *Methyl H^2 -3 β -Acetoxy-16,17-seco-5-androstene-16,17-dioate*. Acidification of the sodium bicarbonate solution from the above separation affords 2.77 g. of a yellow, oily mixture of acids. This acidic material is treated with diazomethane and is then acetylated at room temperature by treatment with acetic anhydride and pyridine overnight. The resulting 3.02 g. of yellow oil is chromatographed on acid-washed alumina, and 598 mg. of semicrystalline material is obtained. After repeated recrystallization from methanol, 91 mg. of methyl H^2 -3 β -acetoxy-16,17-seco-5-androstene-16,17-dioate, m.p. 153.5–154°, $[\alpha]_D^{29} -82.2^\circ$ (chloroform), is obtained.

B. Notes

1. The following compounds were prepared in a degradative study to determine the isotopic distribution in H^2 -cholesterol. According to the deuterium content of the products, 40% of the isotope was at C-6, 3% at C-3 and 50% at or among C-24, C-25, C-26 and C-27. Also see H^2 -3-cholestanone.

2. For general information regarding the Oppenauer oxidation, see *Organic Reactions*.¹

3. A portion of the oil from the oxidation was equilibrated with a refluxing mixture of 40 ml. of methanol, 8 ml. of water and 24 ml. of 5% methanolic potassium hydroxide for 2 hours. This treatment reduced the deuterium content from 1.55 to 1.34 gram atoms per mole.

C. Other Preparations

H^2 -4-Cholesten-3-one has been prepared² from H^2 -3-cholestanone by the method of Rosenkranz.³ The product, m.p. 81–82°, ϵ_{2420} 16,400 (etha-

nol), was purified by: chromatography on alumina, equilibration with methanolic potassium hydroxide, a second chromatographic separation, two recrystallizations from methanol, and one recrystallization from acetone. A purer product, m.p. $82-83^{\circ}$, ϵ_{2420} 16,200 (ethanol), was obtained by preparation of the hydrazone derivative with Girard Reagent T.⁴ The isotope was shown to be attached at C-7. H^2 -4-cholesten-3-one has been obtained⁵ as a by-product in the preparation of H^2 -cholesterol by exchange; and by direct exchange⁶ of 4-cholesten-3-one with a water- H_2^2 -acetic- H_3^2 acid- H^2 medium.

H^2 -Cholesteryl acetate, m.p. $114.5-115^{\circ}$, has been prepared² essentially according to the procedure described.

¹*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 207.

²D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **77**, 139 (1955).

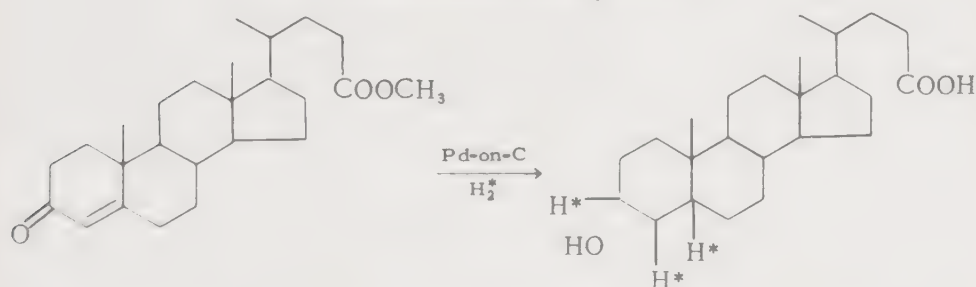
³G. Rosenkranz, O. Mancera, J. Gatica and C. Djerassi, *ibid.*, **72**, 4077 (1950).

⁴*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 85.

⁵K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943); H. S. Anker and K. Bloch, *J. Am. Chem. Soc.*, **66**, 1752 (1944).

⁶D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 871 (1952).

3 α -HYDROXYCHOLANIC-3 β ,4,5- H_3^2 ACID (Lithocholic-3,4,5- H_3^2 Acid)



W. H. Pearlman, M. R. J. Pearlman and S. Elsey, *J. Am. Chem. Soc.*, **71**, 4126 (1949).

A. Procedure

Methyl 3-oxo-4-cholelenoate, 8.56 g., (Note 1) is dissolved in 400 ml. of absolute ether, maintained at 5° , and shaken with 3.5 g. of 5% palladium-on-carbon catalyst (Note 2) in 1 atmosphere of hydrogen- H_2^2 . The reduction product (Note 3) is dissolved in 400 ml. of absolute ether and treated with hydrogen- H_2^2 as above but in the presence of 4.0 g. of platinum oxide catalyst. The final reduction mixture is separated into digitonin precipitated (a) and non-digitonin precipitated (b) fractions according to the procedure described by Schoenheimer and Berliner.¹ The latter fraction (b), 5.51 g., is dissolved in 3 ml. of benzene plus 21 ml. of petroleum ether (b.p. $35-45^{\circ}$) and adsorbed on a column containing 40 g. of aluminum oxide. The product, eluted with mixtures of benzene (5-100%) and petro-

leum ether (Note 4), is crystallized from aqueous methanol to obtain a total of 3.51 g. of crystals melting from 120 to 125°. This material is refluxed for two hours with 5% potassium hydroxide in 90% methanol. The solution is poured into water, acidified and extracted with ether. After the residue from the ether is twice recrystallized from ethyl acetate, the yield of lithocholic-3,4,5-H₃² acid is 2.71 g., m.p. 186-187°. An additional 0.34 g. of crystals, m.p. 185-185.5° is obtained from the mother liquor.

From the digitonin-precipitable material (2.96 g.) is obtained, on treatment with ether-petroleum ether, 740 mg. of crystals, m.p. 139-144°. The material in the mother liquor, which gives crystalline mixtures, is dissolved in 1.5 ml. of benzene plus 8 ml. of petroleum ether, adsorbed on a column containing 25 g. of aluminum oxide and eluted with benzene (10-100%)-petroleum ether and finally with methanol. The benzene (10-20%)-petroleum ether eluates afford, on repeated crystallization from aqueous methanol, 380 mg. of material melting at 108.5-110.5°. This material is hydrolyzed as above and crystallized from ethanol to obtain 359 mg. of 3 β -hydroxycholan-3 α ,4,5-H₃² acid, m.p. 175-177.5°. The benzene (50-100%)-petroleum ether eluates, worked up individually and crystallized from aqueous methanol, give a total of 224 mg. of crystals melting from 141 to 148°. This material is combined with the 740 mg. of ether-petroleum ether precipitated material, m.p. 139-144°, and hydrolyzed as above. On repeated crystallization of the product from ethanol, 604 mg. of 3 β -hydroxyallocholan-3 α ,4,5 α -H₃² acid, m.p. 219-220°, is obtained.

B. Notes

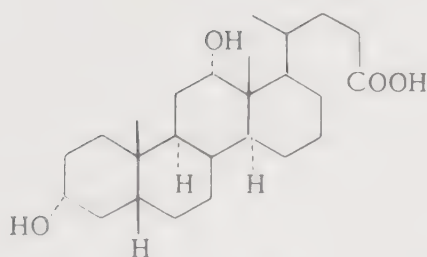
1. Methyl 3-oxo-4-cholenate, m.p. 124-125°, was obtained by Oppenauer oxidation¹ of methyl 3 β -hydroxy-5-cholenate.

2. The catalyst was previously treated with hydrogen-H₂².

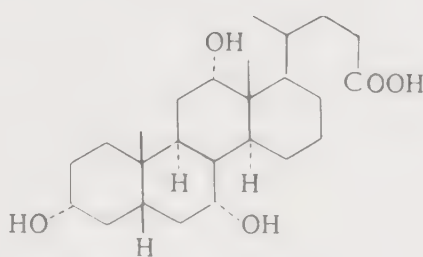
3. The first reduction product appeared to consist mainly of methyl 3-oxocholanate-4,5-H₂² and some methyl 3-oxoallocholanate-4,5-H₂².

4. The eluates were worked up individually.

¹R. Schoenheimer and F. Berliner, J. Biol. Chem. 115, 19 (1936).

H²-CHOLIC ACID

(a)



(b)

N. R. Trenner, H. L. Pfluger, E. G. Newstead, S. L. Jones and C. T. Sutton, J. Am. Chem. Soc., 76, 1196 (1954).

A. Procedure

(a) *H²-Deoxycholic Acid*. In a special reaction vessel (Note 1) is placed a mixture of 10 g. of pure deoxycholic acid (m.p. 174–175°), 50 ml. of water-H₂ containing 2 g. (2.05 equiv.) of sodium hydroxide (Note 2) and the platinum catalyst from 1.5 g. of platinum oxide (Note 3). After the introduction of a glass-covered magnetic stirring bar, the reaction cylinder is flushed with nitrogen and sealed. With stirring, the reaction mixture is heated at 124° for 7 days. After removal of the catalyst by centrifugation, the solution is diluted with 125 ml. of distilled water and then is acidified with hydrochloric acid. The precipitate of H²-deoxycholic acid is collected, washed with water until chloride-free and dried *in vacuo* at 25°. The product is twice recrystallized from acetone; 4 g. of decolorizing carbon is used the first time. After the crystalline product is dried *in vacuo* at 155°, the yield of H²-deoxycholic acid, m.p. 173–174°, is 3.3 g. (Note 4).

(b) *H²-Cholic Acid* (Note 5). In a stainless steel autoclave, 30 g. of cholic acid is dissolved in 180 ml. of water-H₂ containing 6.5 g. (2.3 equiv.) of sodium hydroxide. The catalyst, 7 g. of 10–13% platinum-on-charcoal catalyst, is added, and the mixture is stirred at 115° under nitrogen for 2 days. After the mixture is cooled, the catalyst is removed by filtration. The filtrate is acidified with 75 ml. of 2.5 N hydrochloric acid, and 10 g. of sodium sulfate is added. This solution is extracted 3 times with an equal volume of a mixture of isopropyl alcohol and ether (1:2). The combined alcohol-ether extract is washed with water, dried over sodium sulfate and evaporated to dryness (Note 6). The residue, a brown, gummy mass, is dissolved in acetone and treated with an excess of fresh diazomethane (about 7 g.) in ether. When the esterification is complete, excess ether is removed and the solution is diluted to 1 liter

with acetone. The crude product was adsorbed, from the acetone solution, on a chromatographic column containing 400 g. of dry alumina (Note 7). The column is then washed with about 3.5 liters of acetone to remove impurities (Note 8). The methyl H^2 -cholate is then eluted with about 2.5 l. of absolute methanol which is evaporated to 100 ml., seeded with pure methyl cholate and crystallized at 5° for 24 hours. The crystalline ester (4.5 g.) is dissolved in 100 ml. of 0.8 M aqueous sodium hydroxide solution and heated for 1 hour on a steam-bath. The resulting solution is acidified with 50 ml. of 2.5 N hydrochloric acid and, after addition of 5 g. of sodium sulfate, is extracted with an equal volume of isopropyl alcohol-ether (1:3). The ether phase is washed with water, dried over sodium sulfate and evaporated to dryness. The residue is crystallized from acetone to obtain 3.6 g. of H^2 -cholic acid, m.p. $199-204^\circ$.

B. Notes

1. The reaction vessel was a narrow-necked 100-ml. Pyrex cylinder which fitted by means of a ground glass joint into a vapor jacket equipped with side arms for a boiler and a condenser. By using a compound of the desired boiling point, the temperature of the reaction mixture was maintained at 124° .

2. By the use of an exchange medium containing sodium hydroxide, as many as 8 atoms of stably bound deuterium per molecule of deoxycholic or cholic acid were introduced.

3. The platinum catalyst was prepared by reduction of platinum oxide, in water suspension, with hydrogen. The reduced catalyst was washed several times with water- H_2^2 to remove water and adsorbed hydrogen.

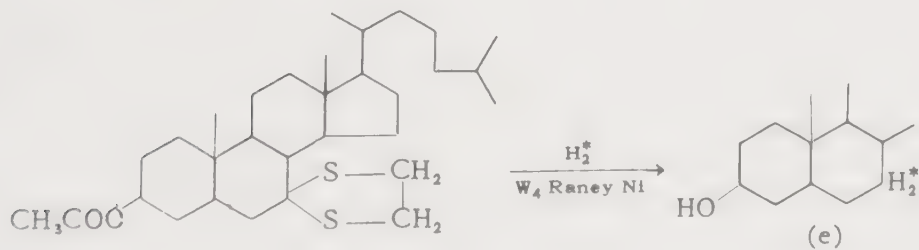
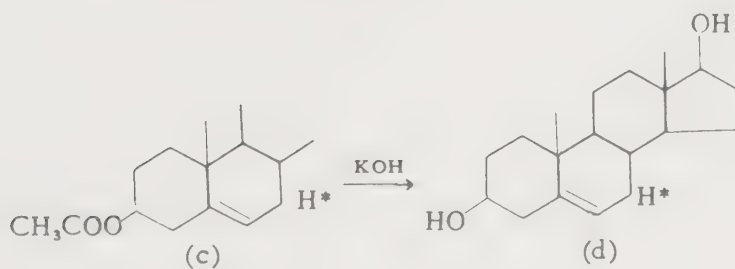
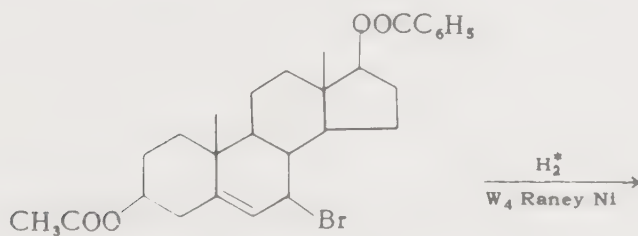
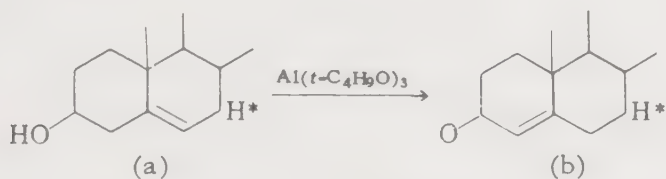
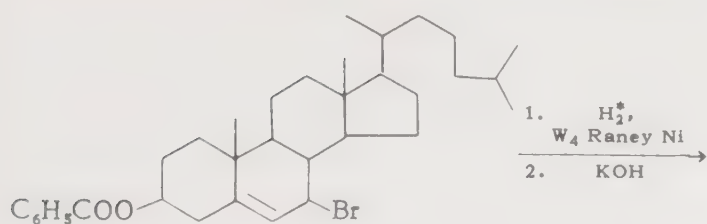
4. The deuterium content was unchanged by heating the product under reflux for 30 minutes with 5% methanolic potassium hydroxide solution.

5. Cholic acid was found to be considerably more susceptible to degradation, under the conditions of the exchange reaction, than deoxycholic acid. Whereas only small amounts of by-products were found in the reaction with deoxycholic acid, in the case of cholic acid, side reactions consumed nearly half of the starting material. A more elaborate purification process was therefore required.

6. Small amounts of acetone were added periodically to facilitate removal of isopropyl alcohol.

7. The alumina had been acid-washed to neutrality.

8. Successful removal of impurities was indicated by the fact that crystalline cholic acid could be obtained easily from the last 50 ml. of acetone wash by evaporating it to dryness and rubbing the residue with a glass rod. The rate of flow through the column, during both the charging and washing operations, was not greater than 3 ml. per min.

5-ANDROSTENE-3 β ,17 β -DIOL-7-H $_2$ 

cooled, acidified with 10% sulfuric acid solution and steam-distilled for 3.5 hours. The residue is extracted with ether, and the combined extract is washed with sodium carbonate solution and water, dried and evaporated to obtain 371 mg. of yellow oil. The oil is chromatographed on alumina and 265 mg. of crystalline product is eluted with ligroin (b.p. 60°). Recrystallization from methanol yields 4-cholesten-3-one-7- H_1^2 , m.p. $79.5-80.5^{\circ}$ (Note 3).

(c) *3 β -Acetoxy-17 β -benzoyloxy-5-androstene-7- H_1^2* . *3 β -Acetoxy-17 β -benzoyloxy-7-bromo-5-androstene*, 543 mg., in 75 ml. of ethyl acetate is reduced with "deuterized" Raney nickel (see Note 1) and hydrogen- H_2^2 at room temperature and atmospheric pressure. The reduction mixture is treated as described for cholesterol-7- H_1^2 to obtain 459 mg. of bromine-free oil which is dissolved in ligroin and chromatographed on acid-washed alumina. From the benzene-ligroin (9:1, 4:1 and 1:1) eluates 311 mg. of crystalline material is obtained. Recrystallization from ether yields a mixture of plates and needles. The two types of crystals are separated by fractional crystallization from acetone; the needles (30 mg., m.p. $303-308^{\circ}$) being less soluble in this solvent. The more soluble plates are then recrystallized several times from ligroin (b.p. 90°) to obtain 189 mg. (41%) of *3 β -acetoxy-17 β -benzoyloxy-5-androstene-7- H_1^2* , m.p. $174-178^{\circ}$; $[\alpha]_D^{28} -4.8 \pm 3^{\circ}$ (8.20 mg. in 2.00 ml. of chloroform).

(d) *5-Androstene-3 β ,17 β -diol-7- H_1^2* . Hydrolysis of the above ester yields *5-androstene-3 β ,17 β -diol-7- H_1^2* , m.p. $180-181^{\circ}$.

(e) *Cholesterol-7- H_2^2* . A solution of 500 mg. of 7,7-(ethylenedithio)-5-cholesten-3 β -yl acetate (Note 4) in 75 ml. of ether is stirred with 5 ml. of "deuterized" Raney nickel (Note 1) for 7 hours. After the mixture stands at room temperature for an additional 16 hours, the catalyst is removed by filtration and is washed several times with ethyl acetate. Evaporation of the combined ether solution and ethyl acetate washings leaves 408 mg. of oil which is hydrolyzed by heating under reflux for 30 minutes with 4 ml. of methanolic potassium hydroxide solution, 25 ml. of methanol and 1 ml. of benzene. The crystalline product is chromatographed on alumina and three main fractions are obtained. Fraction I, 31 mg. of oil eluted with ligroin, is probably the hydrocarbon produced by hydrogenolysis of the 3-acetoxy group. Fraction II, eluted with benzene-ligroin (1:1), is 171 mg. of crystalline cholesterol-7- H_2^2 , m.p. $138-142^{\circ}$. After recrystallization from acetone, this product melts at $146.5-148.5^{\circ}$; $[\alpha]_D^{25} -38.1 \pm 3^{\circ}$ (6.30 mg. in 2.00 ml. of chloroform). Fraction III, eluted with benzene and ether, is 108 mg. of unchanged starting material (Note 5).

(f) *3 β -Acetoxy-17 β -benzoyloxy-5-androstene-7- H_2^2* . A solution of 625 mg. of *3 β -acetoxy-17 β -benzoyloxy-7,7-(ethylenedithio)-5-cholestene* (Note 6) in 100 mg. of anhydrous ether is stirred with 10 ml. of "deuterized" Raney nickel for 10 hours and then allowed to stand for an additional 12

hours. The catalyst is removed by filtration and washed with ethyl acetate. Evaporation of the combined ether solution and ethyl acetate washings leaves 466 mg. of crystalline residue. Chromatographic separation of this residue on acid-washed alumina gives a small fraction in the ligroin eluate, 26 mg. of unknown material, m.p. 125–140°. Fraction II, eluted with benzene-ligroin (1:4) and (1:3), is 158 mg. of 3 β -acetoxy-17 β -benzoyloxy-5-androstene-7-H₂², m.p. 168–174°. This compound is recrystallized from acetone in heavy plates, m.p. 178–179°; $[\alpha]_D^{25}$ -4.9 \pm 2° (10.6 mg. in 2.00 ml. of chloroform). Fraction III, eluted with benzene-ligroin (1:1), benzene and ether, is 216 mg. of nonhomogeneous material, which affords 14 mg. of the desired product and 120 mg. of unchanged thioketal, m.p. 215–221°.

(g) 5-Androstene-3 β ,17 β -diol-7-H₂². This diol is obtained by hydrolysis of the above ester.

B. Notes

1. The preparation of "deuterized" Raney nickel by exchange with water-H₂² is described by Fukushima, *et al.*

2. Infrared analysis of these products revealed the presence of the characteristic absorption at 2100 cm⁻¹ associated with the C-H² bond.

3. The deuterium content of the product remained unchanged after heating 2 hours under reflux in methanolic potassium hydroxide.

4. Preparation of this compound is described by Fukushima, *et al.*

5. The conditions of desulfurization determined the relative amounts of material in each fraction. The yield of hydrocarbon fraction could be decreased by lowering the temperature, but when this was done, the amount of recovered thioketal was large. By carrying out the reaction at room temperature for long periods of time, the best yield of desulfurized product (fraction II) was obtained.

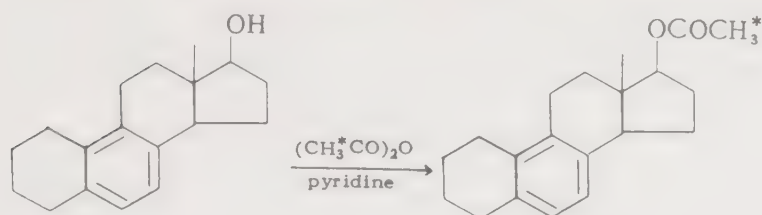
6. Preparation of this compound is described by Fukushima, *et al.*

C. Other Preparations

Cholesterol-7-H₁² has also been prepared by Fukushima, *et al.*, by the reduction of 7-bromocholesteryl benzoate with hydrogen-H₂² and a 5% palladium-on-calcium carbonate catalyst. Hydrolysis of the resulting ester and chromatographic purification of the product gave cholesterol-7-H₁² in 52.5% yield.

¹J. A. K. Buisman, W. Stevens and J. vander Vliet, *Rec. trav. chim.*, 66, 83 (1947).

²S. Bernstein, L. J. Binovi, L. Dorfman, K. J. Sax and Y. Subbarow, *J. Org. Chem.*, 14, 433 (1949).

5,7,9-ESTRATRIEN-17 β -YL ACETATE-H $_3^2$ 

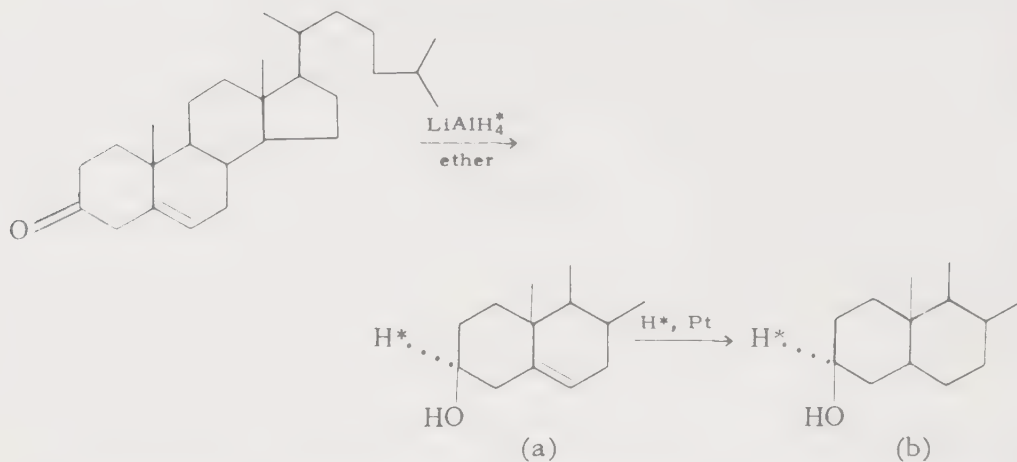
B. Nolin and R. N. Jones, *Can. J. Chem.*, **30**, 727 (1952).

Procedure

In this general procedure, the steroid alcohol (10 to 20 mg.) is dissolved in dry pyridine (0.2 ml.), an equal volume of bis(acetic-H $_3^2$) anhydride is added, and the mixture is kept at room temperature overnight. The excess anhydride and pyridine are removed *in vacuo*, and the residual product is purified by crystallization or sublimation under high vacuum. The sterol acetate-H $_3^2$ esters prepared in this manner are given in the following table.

TABLE XVI, 10
Sterol Acetate-H $_3^2$ Esters

Sterol	Acetate-H $_3^2$ m.p., °C.	Normal Acetate m.p., °C.
5,7,9-Estratrien-17 β -ol	98.0-101.0
Estrone	122.4-124.4	125.2-126.2
3 α -Androstanol	133.6-134.6	133.2-134.0
3 β -Androstanol	85.5-86.4	85.0-86.2
17 β -Androstanol	75.5-77.5	77.3-79.3
20 α -Pregnanol	127.3-127.8
3 β -Hydroxy-5-pregnen-20-one	141.3-142.5	142.5-144.7
3 β -Hydroxy-5-pregnen-20-one-17,21-H $_4^2$	140.2-141.9	142.5-144.7
Cholesterol	112.7-114.4	114.2-114.8
6,8-Cholestadien-3 β -ol	100.0-102.0
3 β -Ergostanol	143.9-144.6	142.4-143.4
14-Ergosten-3 β -ol	87.0-88.0	90.0-91.5
"5-Isoergost-22-en-3 α -ol"	108.9-110.2	106.6-109.4

3 β -CHOLESTANOL-3-H²

R. S. Rosenfeld, D. K. Fukushima, L. Hellman and T. F. Gallagher, *J. Biol. Chem.*, **211**, 301 (1955).

A. Procedure

(a) *Cholesterol-3-H²*. A solution of 20 g. of 5-cholesten-3-one in 100 ml. of ether is added slowly to a suspension of 2.5 g. of lithium aluminum hydride-H₄^{*} in 150 ml. of dry ether. After this mixture is stirred for 3 hours, the excess reagent is destroyed with ethyl acetate. The ether solution is washed with dilute acid and the solvent is evaporated. The residue is dissolved in 250 ml. of ethanol which contains 4 ml. of concentrated hydrochloric acid. The resulting solution is refluxed for 30 minutes and diluted with ether. The latter solution is washed successively with water, alkali and water. After the solution is dried over sodium sulfate, evaporation of the solvent gives 19 g. of crystalline product. The crude product is chromatographed on silica gel. The yield of cholesterol-3-H², after recrystallization from acetone, is 13 g.; m.p. 149–149.5° (Note 1).

(b) *3 β -Cholestanol-3-H²*. Hydrogenation of 440 mg. of cholesterol-3-H², dissolved in a mixture of 30 ml. of acetic acid and 5 ml. of cyclohexane, with 100 mg. of platinum oxide catalyst yields 3 β -cholestanol-3-H², m.p. 141.5–143° (Note 2).

B. Notes

1. From the chromatogram was also obtained 0.75 g. of epicholesterol-3-H²; m.p. 140–141° after recrystallization from acetone.

2. Proof of the location of the isotope at C-3 was obtained by the oxidation of 3 β -cholestanol-3-H² with 2% chromic oxide in acetic acid for 2 hours at room temperature. 3-Cholestanone was obtained.

H²-CHOLESTEROL

K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943). H. S. Anker and K. Bloch, *J. Am. Chem. Soc.*, **66**, 1752 (1944).

A. Procedure

(a) *H²-Cholesterol*. Platinum oxide catalyst, 1.25 g., is suspended in a mixture of 40 ml. of acetic acid-H² and 13 ml. of water-H₂² (Note 1), and reduced with ordinary hydrogen. The excess hydrogen in the flask is replaced by nitrogen, and 12.5 g. of cholesterol is added to the mixture. The reaction flask is then cooled in Dry Ice, evacuated, sealed and shaken for 3 days at 127° (Note 2). The solvent is distilled off *in vacuo*. The residue, which contains appreciable amounts of H²-cholesteryl acetate, is dissolved in ether, filtered, concentrated to dryness and treated for 4 days at room temperature with 400 ml. of 95% ethanol containing 8 g. of potassium hydroxide. After the alcoholic solution is cooled, the crystalline product is collected, and a second crop of crude product is obtained by concentrating the filtrate. By dilution of the mother liquor with water and extraction with ether, a third crop of product is obtained. The combined crude sterol fractions (7.5 g.) are recrystallized several times from acetone. The yield of H²-cholesterol, m.p. 148° (cor.), $[\alpha]_D^{26} -39^\circ$ (2% in chloroform), is 4.9 g. (39.2%). From the combined mother liquors, more sterol is precipitated by digitonin, regenerated and purified *via* the dibromide. The resulting 0.6 g. of H²-cholesterol, m.p. 147.5° (cor.) makes the total yield 5.5 g. (44%) (Note 3).

(b) *H²-3-Coprostanone* (Note 4). In order to separate the residue from the combined mother liquors into alcoholic, ketonic and hydrocarbon fractions, it is first treated with succinic anhydride in pyridine. An additional 1.1 g. of alcoholic material is obtained (Note 5) as the hydrogen succinate derivative. After the removal of pyridine, the remaining material is dissolved in 95% alcohol and refluxed for 2 hours with *p*-hydrazinobenzoic acid and a few drops of acetic acid according to the procedure of Anchel and Schoenheimer.¹ The mixture of *p*-carboxyphenylhydrazones is decomposed¹ by refluxing with pyruvic acid for 4 hours in 95% alcohol. The resulting solution is distributed between 4% potassium carbonate solution and ether. From the ether extract is obtained 2.2 g. of mixed ketones which are then adsorbed on a column of activated aluminum oxide. By fractional elution with petroleum ether-benzene (8:2), 35 mg. of H²-3-coprostanone, m.p. 58–60°, is obtained.

(c) *H²-3-Cholestanone*. Further elution of the adsorbed material with benzene gives 60 mg. of H²-3-cholestanone, m.p. 128–128.5°.

(d) *H²-4-Cholesten-3-one*. Finally, elution with petroleum ether-acetone (19:1) affords 30 mg. of H²-4-cholesten-3-one, m.p. 77–78°.

(e) *H²-Cholestane*. A solution of the non-ketonic material, 0.8 g., in about 20 ml. of petroleum ether is passed through a column of activated aluminum oxide; 70 mg. of *H²-cholestane*, m.p. 74–76°, is obtained.

B. Notes

1. The acetic acid-*H²* contained 60 atom per cent deuterium, and the water-*H₂²* was 99% pure.

2. Bloch and Rittenberg used an oven for heating the flask. A calibrated heating mantle serves quite well according to Gould.²

3. When suspensions of cholesterol in water-*H₂²* were heated in the presence of active platinum, no exchange took place, even at 200°. Increasing the solubility of cholesterol in the medium by the addition of alcohol was without effect. In the platinum catalyzed exchange with mixtures of water-*H₂²*, the amount of deuterium introduced into the sterols increased with decrease in acetic acid concentration from 100% to 70% at 127°, with a corresponding increase in destruction of cholesterol. The experimental conditions described were optimum over the range studied.

4. Since a considerable amount of the initial cholesterol is altered during the exchange reaction, Anker and Bloch isolated and identified a number of the by-products. They also discuss two possible reaction mechanisms and probable locations of the deuterium in the products.

5. Experiments with non-labeled material indicated this fraction to be largely cholesterol.

C. Other Preparations

The platinum catalyzed exchange of deuterium for hydrogen with sterols in solution in acetic acid-*H²* and water-*H₂²* has been investigated by Fukushima and Gallagher.³ With saturated steroids containing ketone groups, an appreciable amount of stably bound deuterium was incorporated and the steroid was recovered in high yield. With increasing unsaturation or with multiple ketone groups considerably more isotope was incorporated and in some instances high recovery of labeled steroid was possible. Hydroxylated steroids were less suitable for the exchange reaction since dehydrogenation and hydrogenolysis markedly diminished the yield. Acetylation resulted in less destruction without materially altering the exchange. The effects of temperature, catalyst and substrate concentration were investigated. Of these variables, temperature was most important since little exchange was effected below 100°.

The distribution of isotope in *H²-cholesterol*, prepared in accordance with the procedure of Bloch and Rittenberg, has also been investigated.^{3,4}

All the isotope present in the steroid nucleus (46% of total) was found in the vicinity of the 5-en-3-ol system, while the isotope in the side chain was concentrated in the isopropyl group. According to the latter reference, the deuterium in the nucleus resides almost exclusively at C-6 with a small amount at C-3. The deuterium (52%) which was found in the side chain was not definitely located but may be distributed among carbons 24, 25, 26 and 27.

H²-Cholesterol has also been prepared^{5,6} from H²-4-cholesten-3-one which was converted to the 3-enol acetate, m.p. 79-81°, with acetic anhydride and acetyl chloride according to the procedure of Westphal.⁷ The 3-enol acetate was reduced with sodium borohydride^{8,9} and the reaction products were then chromatographed on silica gel to obtain the H²-cholesterol, m.p. 148-149°.

Bell and Thomson¹⁰ have also studied the deuteration of cholesterol by catalytic exchange according to the procedure of Bloch and Rittenberg. They concluded that those parts of the sterol molecule containing only saturated carbon chains contain little deuterium, whereas greater deuteration occurs in the vicinity of the hydroxyl group and the double bonds. For example, H²-ergosterol contained more deuterium per molecule than H²-cholesterol under comparable conditions of exchange. It was also shown that double bond migration occurs, to some degree, during the exchange with the consequent production of isomers of the starting compound (see H²-4-cholesten-3-one).

¹M. Anchel and R. Schoenheimer, *J. Biol. Chem.*, **114**, 539 (1936).

²R. G. Gould, private communication.

³D. K. Fukushima and T. F. Gallagher, *Federation Proc.*, **9**, 174 (1950).

⁴D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, **198**, 861 (1952).

⁵*Idem*, **198**, 871 (1952).

⁶*Idem*, *J. Am. Chem. Soc.*, **77**, 139 (1955).

⁷U. Westphal, *Ber.*, **70**, 2128 (1937).

⁸B. Belleau and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 4458 (1951).

⁹W. G. Dauben and J. F. Eastham, *ibid.*, **73**, 4463 (1951).

¹⁰J. Bell and S. J. Thomson, *J. Chem. Soc.*, 1952, 576.

H²-ERGOSTEROL

J. Bell and S. J. Thomson, *J. Chem. Soc.*, 1952, 576.

A. Procedure (Note 1)

A mixture of 2.7460 g. of acetic acid, 0.6975 g. of water-H₂, 0.2075 g. of water and 0.2320 g. of platinum oxide catalyst¹ is weighed into a 6 × 0.5 inch tube. Hydrogen is passed into the suspension for 90 minutes, followed by nitrogen for 60 minutes (Note 2). Then 0.7850 g. of

ergosterol is added, the mixture in the tube is frozen, and the tube is evacuated and sealed. The tube is shaken for 3 days at $123-133^{\circ}$. The acetic acid and water are recovered by distillation *in vacuo* (Note 3). The residue is warmed with ether, the catalyst is filtered off, and the filtrate is evaporated to dryness. H^2 -5,22-Ergostadien- 3β -yl acetate is hydrolyzed by refluxing the residue with 60 ml. of 5% alcoholic potassium hydroxide for 40 minutes. The alcohol is then evaporated under reduced pressure, and the residue is extracted with water and then with ether. Evaporation of the ether leaves a gummy residue which is crystallized from alcohol-benzene (2:1) to obtain 0.090 g. of product, m.p. $150-151^{\circ}$ in a vacuum (Note 4).

B. Notes

1. The procedure of exchange is similar to that described by Bloch and Rittenberg.²

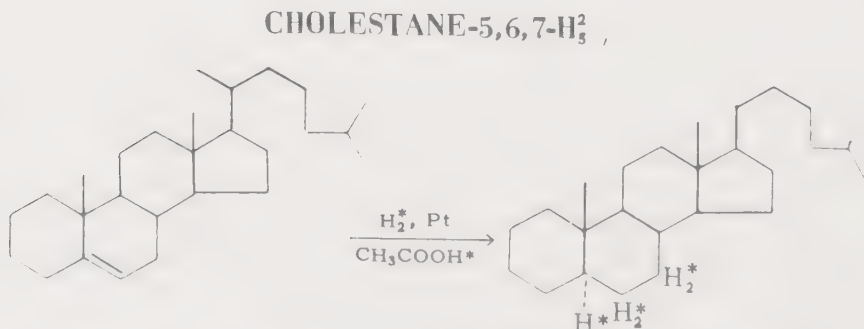
2. The nitrogen was used to remove as much dissolved hydrogen as possible.

3. It was shown that the deuterium content of the sterol and of the acetic acid methyl group were both a function of the amount of catalyst added per gram of these materials. The role of the solvent, acetic acid and water- H_2^2 , was studied and the possibility that exchange of deuterium with the acetic acid methyl group is an essential preliminary to exchange with the sterol was eliminated.

4. An attempted deuteration of ergosterol with glacial acetic acid- H^2 and a platinum-black-on-asbestos catalyst at $123-133^{\circ}$ for 3 days resulted in the introduction of 0.17 atoms per cent deuterium into the sterol.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

²C. Bloch and D. Rittenberg, *J. Biol. Chem.*, 149, 505 (1943).



D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, 77, 139 (1955).

A. Procedure

A solution of 250 mg. of 5-cholestene in 50 ml. of acetic acid- H^2 is hydrogenated with hydrogen- H_2^2 and 50 mg. of platinum catalyst.¹ Recrystallization of the product from acetone gives cholestane-5,6,7- H_3^2 , m.p. 80.5–81.5°, $[\alpha]_D^{25} + 25^\circ$ (chloroform) (Notes 1 and 2).

B. Notes

1. The crystalline product was dissolved in benzene and treated with perbenzoic acid for 24 hours. After chromatographic separation and recrystallization of the product from acetone, the H^2 -cholestane melted at 77–78°.

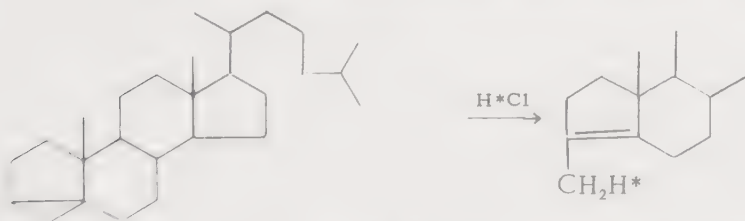
2. It was shown that significantly more than 2 gram atoms of isotope per mole of compound are introduced when various unsaturated steroids are reduced with hydrogen- H_2^2 and a platinum catalyst in acetic acid- H^2 medium. When the double bond was at C-5, it was found that the isotope was distributed between C-5, C-6 and C-7. A mechanism to account for this distribution was proposed. It was shown that exchange as well as reduction occurs at C-6.

C. Other Preparations

H^2 -Cholestane, m.p. 80–80.5°, $[\alpha]_D^{24} + 26.2^\circ$ (chloroform), was also prepared by Fukushima and Gallagher by the reduction of 2-cholestene in a manner similar to that described. This product contained 2.42 gram atoms of hydrogen- H^2 per mole as compared to 2.67 gram atoms per mole in the product from 5-cholestene.

¹*Organic Syntheses*, Coll. Vol. I, 2nd. ed., Wiley, New York, 1941, p. 463.

3-METHYL- H_1^2 -A-NOR-3(5)-CHOLESTENE



D. H. R. Barton, J. E. Page and E. W. Warnhoff, *J. Chem. Soc.*, 1954, 2715.

A. Procedure

A solution of 109 mg. of 3,5-cyclocholestane (Note 1) in 3 ml. of alcohol-free chloroform, containing 4 drops of water- H_2^2 , is stirred with

a magnetic stirrer, and the flask is evacuated. Hydrogen- H^2 chloride (Note 2) is admitted, and the flask is re-evacuated and again filled with hydrogen- H^2 chloride. The flask is sealed and, after the solution is stirred for an additional 15 minutes, it is left overnight (Note 3). The product is chromatographed on alumina. The product, which is eluted with petroleum ether, is recrystallized from chloroform-methanol, m.p. $64-64.5^\circ$, $[\alpha]_D + 54^\circ$ (c, 2.31 in chloroform) (Note 4).

B. Notes

1. This compound, m.p. $79-80^\circ$, $[\alpha]_D + 75^\circ$ (c, 1.42 in chloroform), was prepared according to the procedure of Schmid and Kägi.¹

2. Hydrogen- H^2 chloride was prepared by the addition of distilled phosphorus trichloride to water- H_2^2 .

3. This procedure is analogous to that of Barton and de Mayo.²

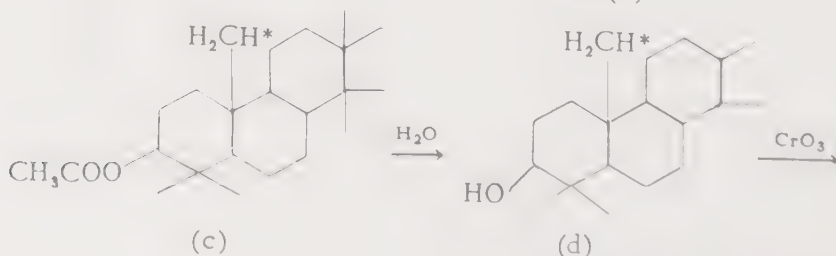
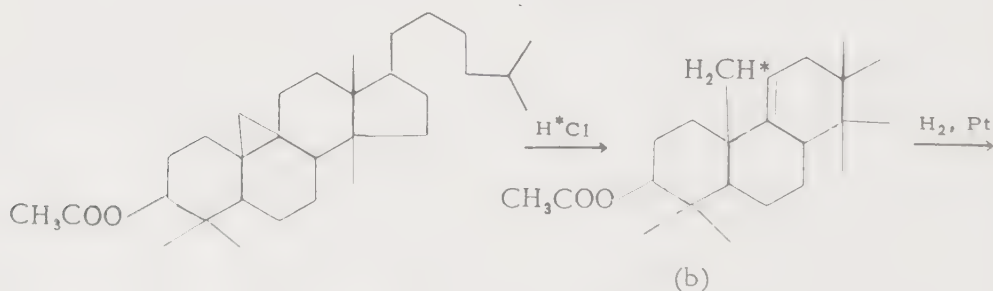
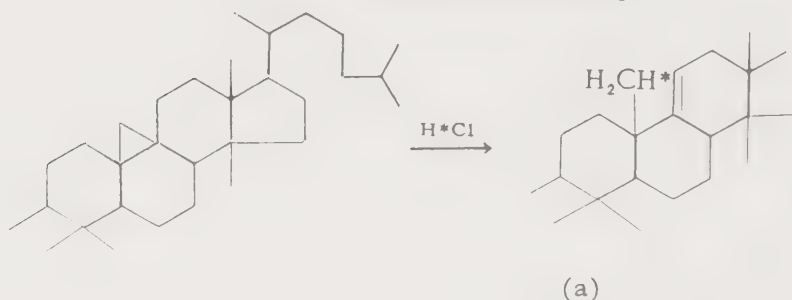
4. Shoppee and Summers³ have also described a synthesis of 3-methyl-A-nor-3(5)-cholestene.

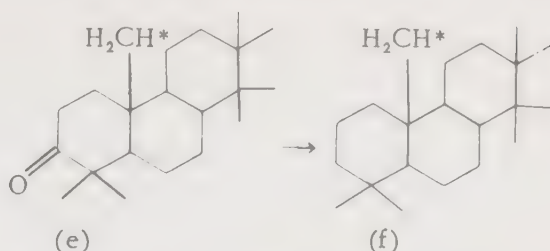
¹H. Schmid and K. Kägi, *Helv. Chim. Acta*, 33, 1582 (1950).

²D. H. R. Barton and P. de Mayo, *J. Chem. Soc.*, 1953, 2178.

³C. W. Shoppee and G. H. R. Summers, *Ibid.*, 1952, 2528.

LANOSTANE-19- H^2





D. H. R. Barton, J. E. Page and E. W. Warnhoff, J. Chem. Soc., 1954, 2715.

A. Procedure (Note 1)

(a) *9(11)-Lanostene-19-H₁²*. According to the procedure of Barton and de Mayo,¹ a solution of 95 mg. of cycloartane in 5 ml. of alcohol-free chloroform, containing 2 drops of water-H₂², is stirred magnetically, and the flask is evacuated. Hydrogen-H₂² chloride (Note 2) is admitted; the flask is re-evacuated and then is filled once more with hydrogen-H₂² chloride. The flask is sealed and, after the solution is stirred for 15 minutes, is left overnight at room temperature. Removal of the solvent *in vacuo* gives a mixture of 9(11)-lanostene-19-H₁², 7-lanostene-19-H₁² and 8-lanostene-19-H₁², m.p. 66–70° (Note 3).

(b) *9(11)-Lanosten-3β-yl-19-H₁² Acetate*. Treatment of 9,19-cyclolanostan-3β-yl acetate with hydrogen-H₂² chloride, as described above, results in the corresponding mixture of 9(11)-lanosten-3β-yl-19-H₁² acetate, 7-lanosten-3β-yl-19-H₁² acetate and 8-lanosten-3β-yl-19-H₁² acetate, m.p. 133–145° (see Note 3).

(c) *3β-Lanostanyl-19-H₁² Acetate*. The mixture of compounds described under (b), above, is dissolved in 50 ml. of acetic acid. After the addition of 200 mg. of platinum oxide catalyst, the mixture is treated at 80° with hydrogen until the ϵ_{\max} at 203 mμ decreased to 1200 (Note 4). The product, 354 mg., is dissolved in a mixture of 25 ml. of acetic acid and 5 ml. of chloroform and treated with 2.5 ml. of 40% peracetic acid. The mixture is left overnight and then chromatographed on alumina. The product is eluted with a mixture of petroleum ether-benzene (3:1). Crystallization of the eluate from chloroform-methanol gives 3β-lanostanyl-19-H₁² acetate, m.p. 147–151°, $[\alpha]_D +39^\circ$ (c, 1.91 in chloroform).

(d) *3β-Lanostanol-19-H₁²*. This compound is obtained by the alkaline hydrolysis of the above ester, (c), (Note 5).

(e) *3-Lanostanone-19-H₁²*. According to the following procedure of Voser, *et al.*,² 450 mg. of 3β-lanostanol is dissolved in a mixture of chloroform and acetic acid and treated with 200 mg. of chromium trioxide. After 15 hours, the product is recrystallized 3 times from dichloromethane-methanol, m.p. 127–128°. The product is sublimed at 120° *in vacuo*, $[\alpha]_D +27^\circ$ (c, 1.27 in chloroform).

(f) *Lanostane-19-H₁²*. The following Wolff-Kishner reduction of 3-lanostanone is the procedure of Voser, *et al.*² To a solution of 250 mg.

of sodium in 10 ml. of absolute alcohol is added 150 mg. of the ketone and 2 ml. of hydrazine hydrate. The mixture is heated for 12 hours at 200° in a sealed tube. The product is then chromatographed on 4.5 g. of aluminum oxide. Elution with petroleum ether affords 130 mg. of product which, after 3 recrystallizations from dichloromethane-methanol, melts at $97-98^{\circ}$; $[\alpha]_D +36^{\circ}$ (c, 1.29 in chloroform). The lanostane-19- H_1^2 prepared in this manner melts at $97-98.5^{\circ}$, after recrystallization from chloroform-methanol; $[\alpha]_D +33^{\circ}$ (c, 1.84 in chloroform) (Note 6).

B. Notes

1. A method for the location of cyclopropane rings in terpenoid and steroid structures is described by Barton, Page and Warnhoff. The method consists in opening the cyclopropane ring with, respectively, hydrogen chloride and hydrogen- H^2 chloride, and comparing the intensities of the C-H bending maxima in the infrared absorption spectra of the two products. In this way the degree of substitution of the carbon to which the proton or deutron attaches itself may be determined. The direction of cyclopropane fission in steroid and terpenoid derivatives appears to be in accordance with the Markownikoff rule. The following compounds were prepared during the elucidation of the structure of cycloartenol, (9,19-cyclo-24-lanosten- 3β -ol).

2. Hydrogen- H^2 chloride was prepared from phosphorus trichloride and water- H_2^2 .

3. According to Bentley, *et al.*,³ treatment of cycloartanol acetate, (9,19-cyclolanostan- 3β -yl acetate), with hydrogen chloride in chloroform results in a mixture comprised of 9(11)-lanosten- 3β -ol (60%) with 7-lanosten- 3β -ol and 8-lanosten- 3β -ol (40%). This mixture melts over a range of $137-157^{\circ}$.

4. This signifies the saturation of all 9(11)-olefinic material.

5. A 5% solution of potassium hydroxide in methanol was usually employed.⁴

6. The latter compound showed no unsaturation when tested with tetranitromethane.

C. Other Preparations

Henry and Spring,⁵ who had independently arrived at the same conclusion regarding the structure of cycloartenol as that proposed by Barton, Page and Warnhoff, have prepared several deuterium-labeled compounds during the course of their investigation. Treatment of 3β -cycloartanyl acetate with hydrogen- H^2 chloride gave 9(11)-lanosten- 3β -yl-19- H_1^2 acetate, m.p. 173° , $[\alpha]_D +84^{\circ}$ (c, 1.2 in chloroform). Oxidation

of this compound with chromic acid gave 3 β -acetoxy-9(11)-lanosten-12-one-19-H², m.p. 183°, [α]_D +90° (c, 0.7 in chloroform).

¹D. H. R. Barton and P. de Mayo, J. Chem. Soc., 1953, 2178.

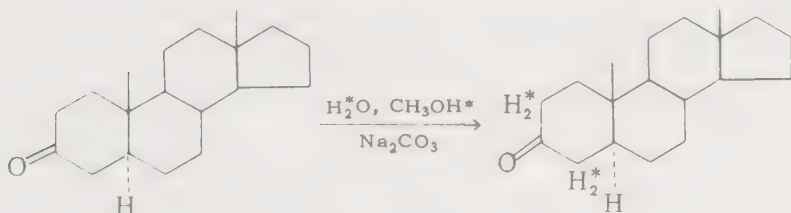
²W. Voser, M. Montavon, Hs. H. Günthard, O. Jeger and L. Ruzicka, Helv. Chim. Acta, 33, 1893 (1950).

³H. R. Bentley, J. A. Henry, D. S. Irvine and F. S. Spring, J. Chem. Soc. 1953, 3673.

⁴C. S. Barnes, D. H. R. Barton, J. S. Fawcett and B. R. Thomas, *ibid*, 1952, 2339.

⁵J. A. Henry and F. S. Spring, Chem. & Ind. (London), 1954, 189.

3-ANDROSTANONE-2,4-H²



B. Nolin and R. N. Jones, Can. J. Chem., 30, 727 (1952).

A. Procedure

3-Androstanone, 25 mg., is dissolved in a mixture of 4 ml. of methanol-H² and 0.5 ml. of water-H² and 5 mg. of anhydrous sodium carbonate is added. After refluxing for 10 minutes, the reaction mixture is evaporated to dryness. This residue is redissolved in a mixture of 4 ml. of methanol-H², and 0.5 ml. of water-H², and the cycle is twice repeated. Finally the product is extracted into anhydrous ether, which is evaporated, and the residue is purified by sublimation under high vacuum. The deuterium-containing ketosteroids prepared in this manner are given in the following table (Note 1).

TABLE XVI, 11

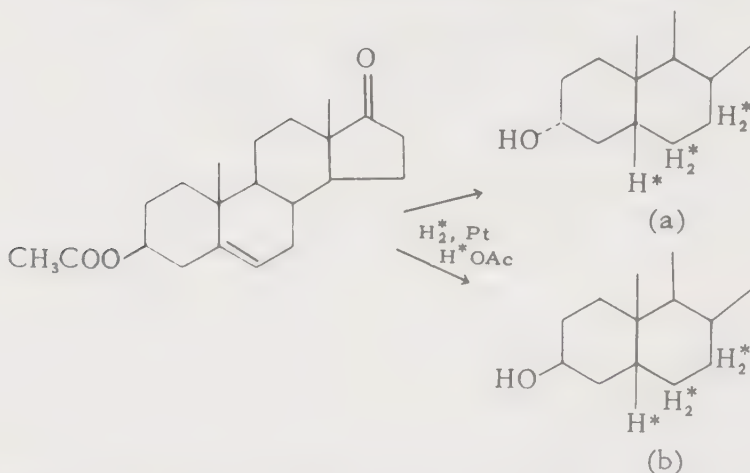
H²-Ketosteroids

Ketosteroid	M.p., °C.	Deuterium atoms per molecule
3-Androstanone	98.7-99.6	3.66
3-Cholestanone	129.1-129.5	3.69
8(14)-Ergosten-3-one	122.5-125.5	3.80
7-Cholestanone	109.0-112.5	1.81
17-Androstanone	121.0-121.8	2.12

B. Notes

1. The 3-ketosteroids underwent deuterium exchange to the extent of 3.7–3.8 atoms of deuterium per molecule, from which it is inferred that the methylene at both C-2 and C-4 participate in the enolization. 17-Androstanone, in which only the C-16 methylene can participate in enolization, exchanged to the extent of 2.12 atoms of deuterium per molecule. When 7-cholestanone was treated with sodium carbonate in methanol- H^2 and water- H^2 , only 0.59 atom of deuterium per molecule was exchanged. The use of sodium hydroxide- H^2 instead of carbonate increased the exchange to 1.81 deuterium atoms per molecule.

3 β -HYDROXY-17-ETIOCHOLANONE-5,6,7- H^2_3



D. K. Fukushima and T. F. Gallagher, J. Am. Chem. Soc., 77, 139 (1955).

A. Procedure

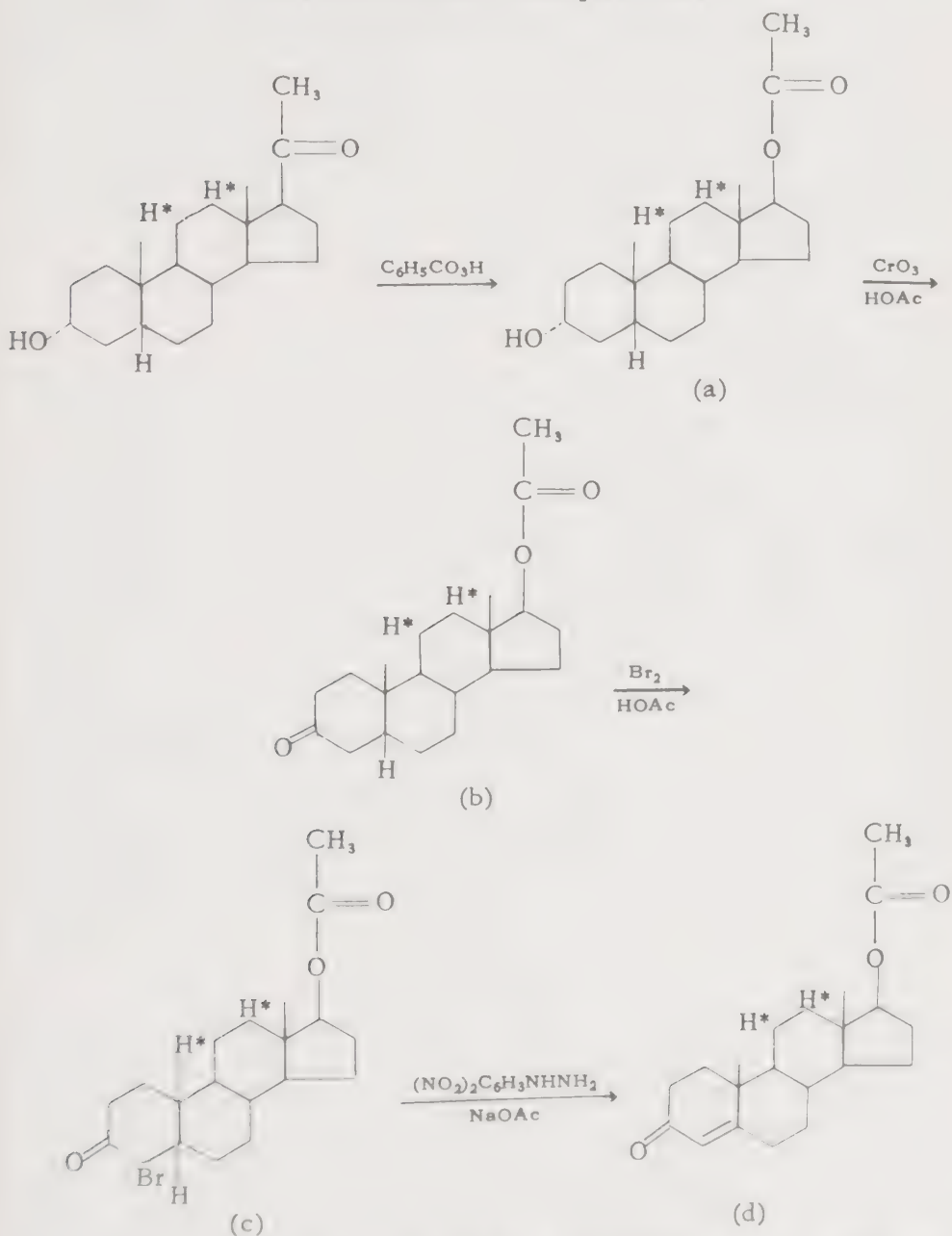
(a) 3 α -Hydroxy-17-etiocholanone-5,6,7- H^2_3 , (H^2_3 -5-Isoandrosterone) (Note 1). A solution of 6.60 g. of 3 β -acetoxy-5-androsten-17-one in 70 ml. of acetic acid- H^2 is hydrogenated with hydrogen- H^2 in the presence of 600 mg. of a platinum catalyst.¹ A part of the reduction product, 2.75 g., is treated with 2.00 g. of *N*-bromoacetamide in 25 ml. of *t*-butyl alcohol, which contains 0.5 ml. each of water and pyridine. The crude product is hydrolysed with methanolic potassium hydroxide solution. Recrystallization of the product from acetone gives 3 α -hydroxy-17-etiocholanone-5,6,7- H^2_3 , m.p. 175.5–176.5°, $[\alpha]_D^{22} + 89.6^\circ$ (CHCl_3).

(b) 3 β -Hydroxy-17-etiocholanone-5,6,7- H^2_3 . The mother liquors from the above preparation are chromatographed on alumina to obtain 3 β -hydroxy-17-etiocholanone-5,6,7- H^2_3 , which is recrystallized from ether, m.p. 154.5–156°, $[\alpha]_D^{22} + 93.7^\circ$ (CHCl_3).

B. Notes

1. See Note 2, Cholestane-5,6,7- H_3^2 .

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

TESTOSTERONE-11,12- H_2^2 ACETATE

B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, *J. Biol. Chem.*, **184**, 393 (1950).

A. Procedure

(a) *3 α -Hydroxyetiocholan-17 β -yl-11,12- H_2^2 Acetate*. 3 α -Hydroxy-20-pregnanone-11,12- H_2^2 (1.0 g.) is dissolved in 4 ml. of formic acid (sp. gr. 1.2) and heated at 70° for 4.5 hours. The excess formic acid is removed under reduced pressure, leaving a residue of 3 α -formoxy-20-pregnanone-11,12- H_2^2 (Note 1). Without purification, the residue is dissolved in 1 ml. of chloroform, and 3.38 ml. of 0.87 M perbenzoic acid in chloroform is added. After 11 days at 25°, no perbenzoic acid remains, and 1.027 g. of an oily product is obtained upon evaporation of the solvent. By separation with Girard's Reagent T, 595 mg. of a crystalline non-ketonic fraction and 406 mg. of a ketonic fraction are obtained. The former is adsorbed on 100 times its weight of aluminum oxide (Note 2) and left for 24 hours. The product is eluted with ether-methanol, and after two recrystallizations from acetone-petroleum ether, 382 mg. of 3 α -hydroxyetiocholan-17 β -yl-11,12- H_2^2 acetate is obtained; m.p. 169.5–170°, $[\alpha]_D^{28} + 18.2^\circ$ (chloroform).

(b) *17 β -Acetoxy-3-etiocholanone-11,12- H_2^2* . 3 α -Hydroxyetiocholan-17 β -yl-11,12- H_2^2 acetate, 240 mg. dissolved in acetic acid, is treated, at 10°, with 55 mg. of chromium trioxide (2.2 equivalents) in glacial acetic acid. The mixture is kept at 10° overnight. The excess chromium trioxide is reduced with ethanol, and the acetic acid is evaporated under reduced pressure. The product is then purified by chromatography on alumina. The yield of 17 β -acetoxy-3-etiocholanone-11,12- H_2^2 is 80%; m.p. 149–151° (Note 3), $[\alpha]_D + 31.5^\circ$ (chloroform).

(c) *17 β -Acetoxy-4-bromo-3-etiocholanone-11,12- H_2^2* . To a chilled solution of 400 mg. of 17 β -acetoxy-3-etiocholanone-11,12- H_2^2 in glacial acetic acid is added dropwise (Note 4) 5.5 ml. of a 0.48 M solution of bromine in acetic acid (2.1 equivalents). After evaporation of the solution to a few ml., at 40° under reduced pressure, ether is added, and a crop of octahedral crystals is obtained. After recrystallization from ether-chloroform, this first fraction, 174 mg. (35%), melts at 185–192°, $[\alpha]_D + 36^\circ$ (chloroform). A second fraction (Note 5), 150 mg. (30%), m.p. 175–180°, is obtained from the mother liquor (Note 6); $[\alpha]_D + 46^\circ$ (chloroform).

(d) *Testosterone-11,12- H_2^2 Acetate*, (17 β -Acetoxy-4-androsten-3-one-11,12- H_2^2). Pure 17 β -acetoxy-4-bromo-3-etiocholanone-11,12- H_2^2 (second fraction, $[\alpha]_D + 46^\circ$), 100 mg., is dissolved in 3 ml. of glacial acetic acid at 60°, and 25 mg. of sodium acetate is added. Under an atmosphere of nitrogen, 60 mg. of 2,4-dinitrophenylhydrazine is dissolved in this solution. The reaction mixture is kept at 60° for 20 minutes; during this time the mono-2,4-dinitrophenylhydrazone of testosterone-11,12- H_2^2 crystallizes. To the resulting suspension, 5 ml. of chloroform, 50 mg.

of sodium acetate, 3 ml. of freshly distilled pyruvic acid and 1 ml. of water are added. The resulting solution is refluxed at about 70° , under an atmosphere of nitrogen, until the cleavage of the dinitrophenylhydrazone is complete, as indicated by a color change. During this period, 3 ml. of pyruvic acid and 2 ml. of water are added in 3 portions as the reaction proceeds. The solution is transferred with about 50 ml. of ether to a separatory funnel, extracted with sodium carbonate solution to remove all of the pyruvic acid dinitrophenylhydrazone, washed twice with 5% sodium hydroxide solution and several times with water, and dried over sodium sulfate. The crystalline residue from the ether solution is sublimed in a high vacuum at 90° . After recrystallization of the sublimate from ether-petroleum ether, the yield is 73 mg. (90%); m.p. $138-140^{\circ}$; $[\alpha]_D +92^{\circ}$ in chloroform, $\epsilon_{2410} = 17,400$ in methanol (Note 8).

B. Notes

1. The absence of free hydroxyl groups in the formoxy compound was indicated by infrared analysis.
2. This aluminum oxide was known to be capable of hydrolyzing esters.
3. A rearrangement occurs at 146° .
4. The bromine solution is added within 15 minutes with the rate determined by the rate of decolorization.
5. The second fraction of 17 β -acetoxy-4-bromo-3-etiocholanone-11,12- H_2^2 was more pure than the first.
6. By debromination of the residue from the last mother liquor with zinc, 20% of the starting material is recovered.
7. The introduction of the 4,5-double bond in testosterone was done according to a modification of the procedure of Mattox and Kendall¹ (see progesterone-11,12- H_2^2).
8. Dehydrobromination of the less pure first fraction of 17 β -acetoxy-4-bromo-3-etiocholanone-11,12- H_2^2 gave, in addition to a 60% yield of testosterone-11,12- H_2^2 acetate, a small amount of 17 β -acetoxy-2,4-dibromo-3-etiocholanone-11,12- H_2^2 , m.p. $187-192^{\circ}$, $[\alpha]_D + 12.5^{\circ}$.

C. Other Preparations

3 α -Hydroxyetiocholan-17 β -yl-11,12- H_2^2 acetate has been prepared² by the perbenzoic acid oxidation of 3 α -hydroxy-20-pregnanone-11,12- H_2^2 . Saponification of the product gave 3 α ,17 β -etiocholanediol-11,12- H_2^2 and a small amount of 17 β -acetoxy-4-hydroxy-3,4-seco-3-etiocholan-11,12- H_2^2 acid lactone, which is a by-product of the oxidation.

H^2 -Testosterone has been prepared³ from H^2 -4-androstene-3,17-dione which was exchanged with a 70% acetic acid- H^2 -water- H_2^2 mixture in the presence of platinum catalyst. Reaction of H^2 -4-androstene-3,17-dione

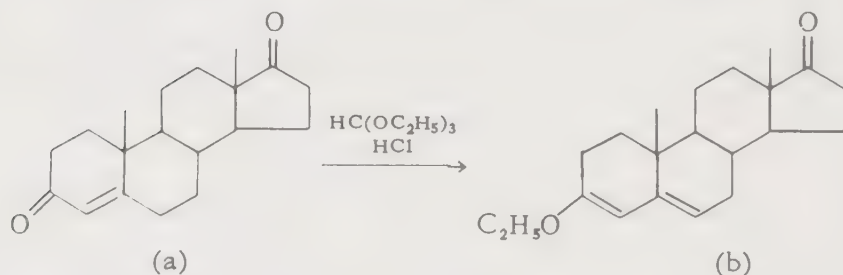
with ethyl orthoformate gave the 3-enol ether of H^2 -4-androstene-3,17-dione, (3-ethoxy-2,4-androstadien-17-one), which was reduced with lithium aluminum hydride to obtain H^2 -testosterone.

¹V. R. Mattox and E. C. Kendall, J. Am. Chem. Soc., 70, 882 (1948).

²B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, J. Biol. Chem., 184, 393 (1950).

³D. K. Fukushima and T. F. Gallagher, *ibid*, 198, 871 (1952).

H^2 -3-ETHOXY-3,5-ANDROSTADIEN-17-ONE



D. K. Fukushima and T. F. Gallagher, J. Biol. Chem., 198, 871 (1952).

A. Procedure

(a) H^2 -4-Androstene-3,17-dione. The procedure used in the exchange reaction is essentially that of Bloch and Rittenberg¹ (see H^2 -cholesterol). In this method, which is general for steroids, 5.50 g. of platinum oxide catalyst² is prereduced with hydrogen- H_2^2 in 125 ml. of 70% acetic- H_3^2 acid- H^2 in water- H_2^2 . To this suspension is added 11.00 g. of 4-androstene-3,17-dione and 140 ml. of 70% acetic- H_3^2 acid- H^2 -water- H_2^2 mixture. The mixture is shaken at 150° for 2 days. After removal of the catalyst, the solvent is distilled off *in vacuo*. Chromatographic separation of the residue on alumina affords 10.05 g. of H^2 -4-androstene-3,17-dione, which melts at 170.5–171.5 after recrystallization from acetone-petroleum ether (Note 1).

(b) H^2 -3-Ethoxy-3,5-androstadien-17-one. The 3-enol ether of H^2 -4-androstene-3,17-dione is prepared from 6.3 g. of H^2 -4-androstene-3,17-dione, m.p. 167–170°, by adaptation of the following method of Serini and Köster.³ To a solution of 2.86 g. of 4-androstene-3,17-dione in 10 ml. of benzene are added 1.7 g. of ethyl orthoformate, 1.4 g. of absolute alcohol and 10 drops of approximately 8% alcoholic hydrogen chloride solution. After heating for 2 hours at 75°, the reaction mixture is evaporated to dryness under vacuum. The crystalline residue is recrystallized from alcohol containing pyridine. The enol ether crystallizes in plates, m.p. 152°; $[\alpha]_D^{20}$ -89° (dioxane) (Note 2).

(c) H^2 -4,16-Pregnadiene-3,20-dione, (H^2 -16-Dehydropregesterone) (Note 3). A mixture of 1.00 g. of 4,16-pregnadiene-3,20-dione, 125 mg. of platinum oxide catalyst² and 6 ml. of 70% acetic- H_3^2 acid- H^2 in water- H_2^2 is heated at 130° for 2 days. After removal of the catalyst and the solvent, the product is refluxed with aqueous base for 30 minutes under a nitrogen atmosphere. The product, 1.04 g. of yellow oil, crystallizes on standing and is chromatographed on alumina (Note 4), to obtain H^2 -4,16-pregnadiene-3,20-dione, m.p. 186.5 – 189° ; $[\alpha]_D^{30} +162^\circ$ (ethanol); ϵ_{2390} 24,900 (ethanol) and H^2 -16 α -methoxyprogesterone, m.p. 131 – 132° ; $[\alpha]_D^{30} +112^\circ$ (ethanol); ϵ_{2400} 15,500 (ethanol).

(d) H^2 -3 β -Hydroxy-5-androsten-17-one, (H^2 -Dehydroisoandrosterone). A mixture of 350 mg. 3 β -hydroxy-5-androsten-17-one, 60 mg. of platinum oxide catalyst² and 5 ml. of 70% acetic- H_3^2 acid- H^2 in water- H_2^2 is heated at 130° for 2 days. After removal of the catalyst and solvent, the product is refluxed with base for 30 minutes. Then, 335 mg. of an oil is isolated (Note 5), which is chromatographed on alumina to obtain 45 mg. (13%) of H^2 -3 β -hydroxy-5-androsten-17-one. After recrystallization from petroleum ether, the product melts at 136 – 137° .

(e) H^2 -3 β -Acetoxy-5-androsten-17-one, (H^2 -Dehydroisoandrosterone Acetate). A mixture of 1.00 g. of 3 β -acetoxy-5-androsten-17-one, 125 mg. of platinum oxide catalyst² and 5 ml. of 70% acetic- H_3^2 acid- H^2 in water- H_2^2 is heated at 130° for 2 days. The exchanged product is refluxed with base for 30 minutes and then chromatographed on alumina to obtain 465 mg. (54%) of crude 3 β -hydroxy-5-androsten-17-one. After recrystallization from petroleum ether, the product melts at 138.5 – 139.5° (Note 6).

(f) H^2 -Estrone. A mixture of 1.00 g. of estrone, 500 mg. of platinum oxide catalyst² and 24 ml. of 70% acetic- H_3^2 acid- H^2 is heated at 130° for 3 days. The product is recrystallized from ethanol and digested with acetone to obtain 482 mg. of H^2 -estrone, m.p. 256 – 256.5° ; ϵ_{2800} 2010 (95% ethanol). An additional 236 mg. of H^2 -estrone, m.p. 235 – 240° is obtained from the mother liquors (Note 7).

B. Notes

1. Equilibration of this product with methanolic potassium hydroxide under reflux reduced the deuterium content from 4.87 to 0.83 g. atom per mole.

2. Fukushima and Gallagher recrystallized the product from acetone and obtained 3.27 g. of enol ether, m.p. 143 – 151° . This material was then used in the preparation of H^2 -testosterone.

3. The following steroids were also prepared by the above general exchange procedure.

4. The product had $\epsilon_{1\text{ cm.}}^{1\%} = 378$ (ethanol) indicating about 75% of 16 α -methoxyprogesterone.⁴

5. This material showed absorption, at 2400 \AA , indicating the presence of 4-androstene-3,17-dione.

6. Oppenauer oxidation of the product, followed by 6 hours of refluxing in base, gave H^2 -4-androstene-3,17-dione, m.p. $170\text{--}171.5^\circ$.

7. A sample of 400 mg. of H^2 -estrone (26.05 atom per cent excess deuterium) was heated in a nitrogen atmosphere under reflux with 40 ml. of 5% methanolic potassium hydroxide and 40 ml. of water for 2 hours. Carbon dioxide was bubbled through the cooled mixture which was then extracted with ethyl acetate. The extract was washed with water, dried, and the solvent was removed. Recrystallization of the residue from methanol gave 304 mg. of H^2 -estrone, m.p. $256\text{--}258^\circ$; $\epsilon_{2810} = 2160$ (95% ethanol); 19.42 atom per cent excess deuterium. The base-equilibrated H^2 -estrone was then heated under reflux with 100 ml. of ethanol and 100 ml. of 4 N hydrochloric acid for 6 hours. Recrystallization of the product from methanol yielded H^2 -estrone, m.p. $257\text{--}259^\circ$; $\epsilon_{2810} = 2050$ (95% ethanol); 14.52 atom per cent excess deuterium. Acetylation of this product at room temperature with pyridine and acetic anhydride gave H^2 -estrone acetate, m.p. $126.5\text{--}127.5^\circ$, $\epsilon_{2680,2750} = 745$ (95% ethanol); $[\alpha]_D^{25} + 136.5^\circ$ (chloroform); 13.55 atom per cent excess deuterium.

¹K. Bloch and D. Rittenberg, *J. Biol. Chem.*, **149**, 505 (1943).

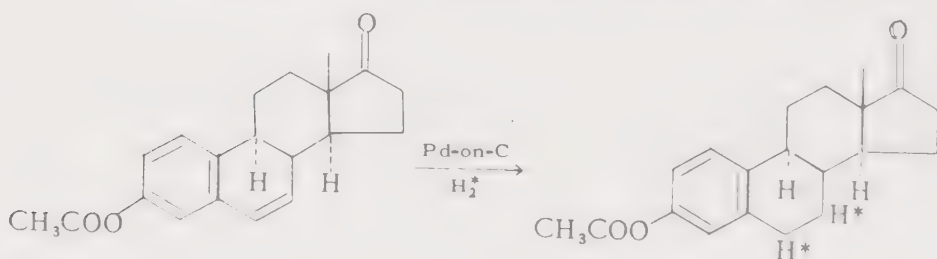
²*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

³A. Serini and H. Köster, *Ber.*, **71**, 1766 (1938).

⁴D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, **73**, 196 (1951).

ESTRONE-6,7- H_2^2 ACETATE

$[\beta\text{-Acetoxy-1,3,5(10)-estratrien-17-one-6,7-}\text{H}_2^2]$



W. H. Pearlman and M. R. J. Pearlman, *J. Am. Chem. Soc.*, **72**, 5781 (1950).

A. Procedure

3-Acetoxy-1,3,5(10),6-estratetraen-17-one, (6-dehydroestrone acetate) (Note 1), 98 mg., is dissolved in 35 ml. of cyclohexane and shaken in an atmosphere of hydrogen- H_2^2 at 25° in the presence of 98 mg. of 5% palladium-on-charcoal (Note 2). After the adsorption of hydrogen- H_2^2 ceases in about 20 minutes, the solution is filtered and the solvent is removed.

The residue is crystallized from alcohol to obtain 91 mg. of product, m.p. 125–126° (Note 3). The product is refluxed for 1.5 hours with 5% potassium hydroxide in 90% methanol, and the solution is then kept at room temperature for 48 hours. After the methanolic solution is diluted with water and acidified, the recovered estrogenic material is dried and treated with acetic anhydride and pyridine for 24 hours. The acetate is adsorbed on a column of 2 g. of aluminum oxide and eluted with petroleum ether-ether (1:1) to obtain 63.5 mg. of colorless material. After crystallization from alcohol, the product melts at 125–126°, $[\alpha]_D^{29} +152 \pm 6^\circ$ (absolute ethanol); ϵ 796, $\lambda_{\text{max}}^{\text{alc.}}$ 270 m μ ; ϵ 438, $\lambda_{\text{max}}^{\text{alc.}}$ 250 m μ .

B. Notes

1. The acetyl derivative, m.p. 139–140°, of 6-dehydroestrone, m.p. 260–262°, was used. 6-Dehydroestrone, first prepared by Pearlman and Wintersteiner,¹ is obtainable in about 40% yield from 1,4,6-androstatriene-3,17-dione by the procedure of Rosenkranz.²

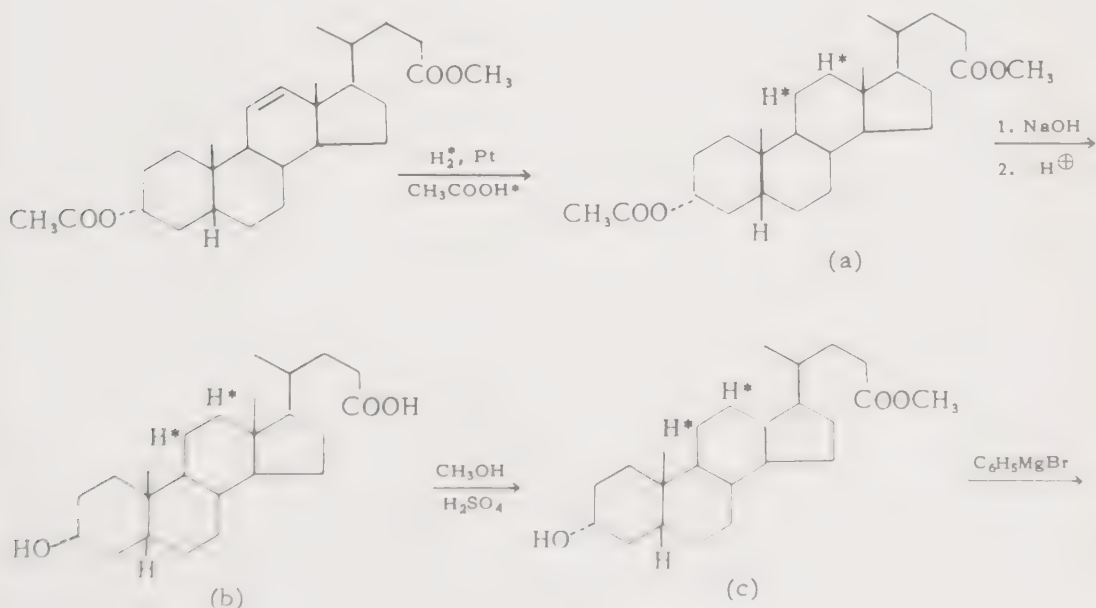
2. The catalyst was previously treated with hydrogen-H₂.

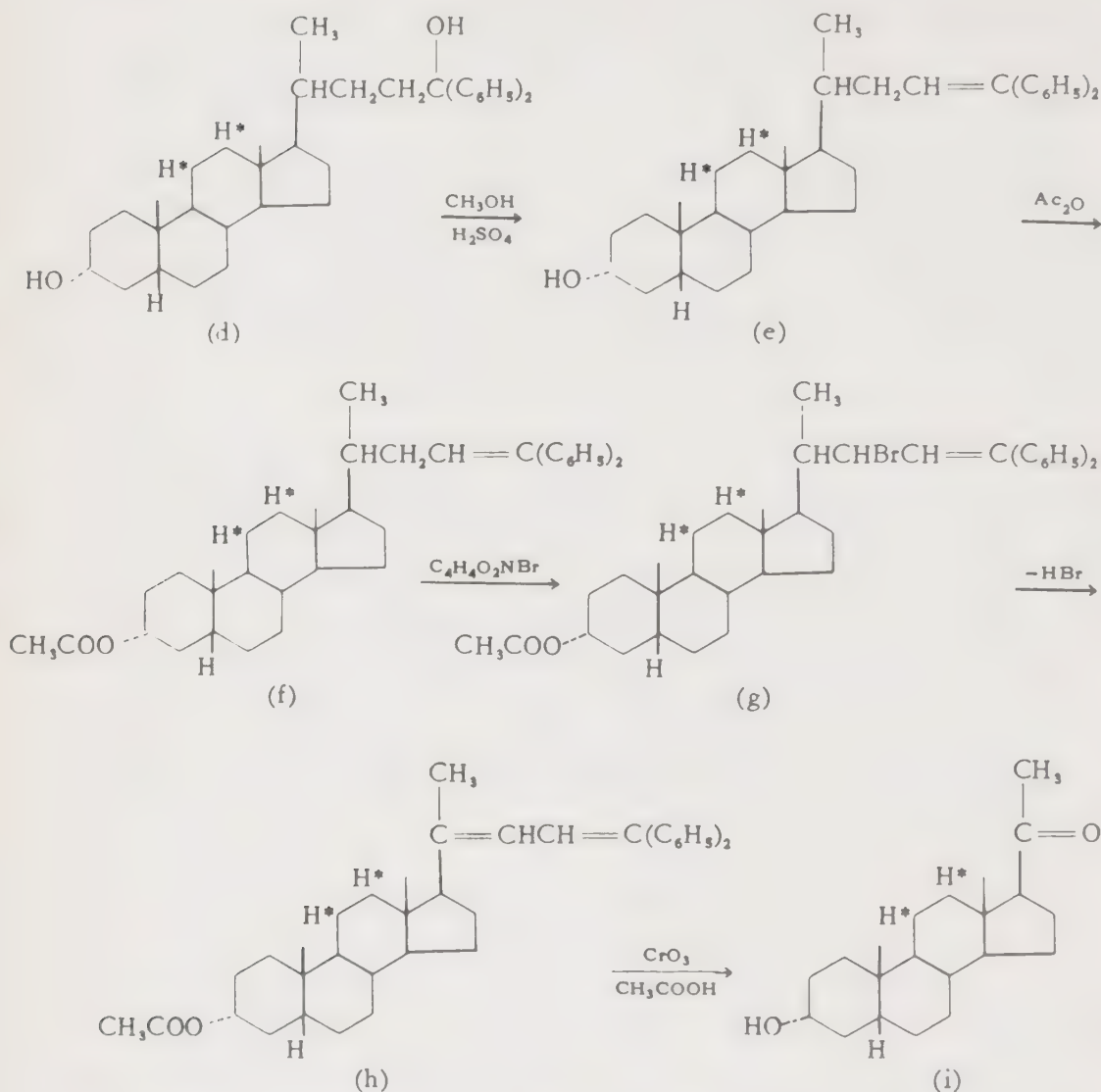
3. The product did not depress the melting point on admixture with estrone acetate, m.p. 125–126°.

¹W. H. Pearlman and O. Wintersteiner, J. Biol. Chem., 132, 605 (1940).

²G. Rosenkranz, C. Djerassi, S. Kaufman, J. Pataki and A. J. Romo, Nature, 165, 815 (1950).

3 α -HYDROXY-20-PREGNANONE-11,12-H₂





B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, J. Biol. Chem., 184, 393 (1950).

A. Procedure

(a) *Methyl 3 α -Acetoxycholanate-11,12- H_2^2* . Methyl 3 α -acetoxycholanate is hydrogenated with hydrogen- H_2^2 , in 10-g. portions, using 2 g. of platinum oxide catalyst in 125 ml. of acetic acid- H^2 solvent. The solution is decanted from the catalyst and filtered quickly; the same catalyst is used to reduce about 150 g. of material. The acetic acid- H^2 is distilled from the product under vacuum and is also used repeatedly (Note 1). The residue is methyl 3 α -acetoxycholanate-11,12- H_2^2 .

(b) *3 α -Hydroxycholan-11,12- H_2^2 Acid, (Litbocholic-11,12- H_2^2 Acid)*. The residue of methyl 3 α -acetoxycholanate-11,12- H_2^2 is saponified by

heating with 1 *N* sodium hydroxide under reflux for 1 hour. After the solution is cooled and acidified, the lithocholic-11,12- H_2^2 acid is collected, washed and dried.

(c) *Methyl 3 α -Hydroxycholanate-11,12- H_2^2 , (Methyl Lithocholate-11,12- H_2^2)*. The above acid dissolved in methanol is esterfied at room temperature in the presence of concentrated sulfuric acid.

(d) *24,24-Diphenyl-3 α ,24-cholanediol-11,12- H_2^2* (Note 2). To 51.8 g. of methyl lithocholate-11,12- H_2^2 in 300 ml. of dry benzene is added an ether solution of phenylmagnesium bromide prepared from 47 g. of magnesium and 312 g. of bromobenzene. The ether is distilled off, and the remaining solution is refluxed for 15 hours, then cooled and poured into ice and hydrochloric acid. The mixture is extracted with chloroform, and the extract is washed with sodium hydroxide solution and water and dried.

(e) *24,24-Diphenyl-23-cholen-3 α -ol-11,12- H_2^2* . After the addition of 100 mg. of iodine to the dry chloroform solution of 24,24-diphenyl-3 α ,24-cholanediol-11,12- H_2^2 , the solvent is distilled off; fresh solvent is added and distilled twice more. During this process water is eliminated from the tertiary carbinol. The mixture is heated with 380 ml. of methanol, containing 13 g. of potassium hydroxide, then treated with steam, and the residue is taken up in ether-chloroform solvent. The solution is washed with water, dried over sodium sulfate and evaporated to dryness.

(f) *3 α -Acetoxy-24,24-diphenyl-23-cholene-11,12- H_2^2* . The residue of 24,24-diphenyl-23-cholen-3 α -ol-11,12- H_2^2 is acetylated with acetic anhydride in the presence of pyridine. After recrystallization from acetone, the product, 47.4 g., melts at 166–167°; $[\alpha]_D^{22} + 64^\circ$ (c, 0.966 in $CHCl_3$).

(g) *3 α -Acetoxy-22-bromo-24,24-diphenyl-23-cholene-11,12- H_2^2* . A mixture of 20 g. of 3 α -24,24-diphenyl-23-cholene-11,12- H_2^2 , 6.61 g. of *N*-bromosuccinimide and 300 ml. of carbon tetrachloride is heated under reflux; after 10–15 minutes hydrogen bromide is evolved. The solution of 3 α -acetoxy-22-bromo-24,24-diphenyl-23-cholene-11,12- H_2^2 is cooled, and succinimide, formed in the reaction, is removed.

(h) *3 α -Acetoxy-24,24-diphenyl-20(22),23-choladiene-11,12- H_2^2* . To the above filtrate is added 30 ml. of dimethylaniline (Note 3), the carbon tetrachloride is distilled off, and the remaining dimethylaniline solution is heated for 10 minutes. The cooled mixture is treated with ether, and the resulting solution is washed with dilute hydrochloric acid and water, dried over sodium sulfate and evaporated. The resulting diene, in 40 ml. of pyridine, is treated with 30 ml. of acetic anhydride at 20° for 15 hours. After the solution is evaporated to dryness *in vacuo*, the residue, dissolved in ether, is washed with dilute hydrochloric acid and water, dried over sodium sulfate and concentrated to dryness. A warm acetone solution of the residue is treated with carbon, filtered and cooled. The first crystalline product obtained, 7.16 g., m.p. 159–163°, $[\alpha]_D^{21} + 76 \pm 4^\circ$ (c, 0.908 in chloroform), is a mixture of the acetoxydiene and some un-

changed acetoxydiphenylcholene (f). Upon standing open to the atmosphere, the mother liquor deposits crystals (5.95 g.) which melt at 100° and contain water of crystallization. After recrystallization either from ethyl acetate or ether-methanol, the water-free diene melts at $166\text{--}168^{\circ}$, $[\alpha]_D^{21} +84 \pm 4^{\circ}$ (c, 0.937 in chloroform).

(i) 3α -Hydroxy-20-pregnanone-11,12- H_2^2 . (Note 4). To a solution of the acetoxydiene in a mixture of 300 ml. of alcohol-free chloroform and 300 ml. of 80% acetic acid is added dropwise, with cooling, a solution 15 g. of chromium trioxide in 300 ml. of 80% acetic acid. The solution, kept at 20° , is then stirred for 3 hours. With good cooling, the excess chromium trioxide is destroyed carefully with sodium sulfite. The solution is evaporated to dryness *in vacuo*, the residue is extracted with ether-chloroform, and the resulting solution is washed with sodium hydroxide solution and water, dried over sodium sulfate and evaporated to dryness. The crude product, which weighs 17.21 g., is separated, with the aid of 20 g. of Girard-T Reagent, into a ketone-free fraction of 4.9 g. and a ketone fraction of 10.3 g. The latter fraction is crystallized from hexane or petroleum ether to obtain 4.12 g. of 3α -hydroxy-20-pregnanone-11,12- H_2^2 , m.p. $151\text{--}154^{\circ}$ (Note 5). The residue from evaporation of the mother liquor, dissolved in benzene, is passed through a column of 60 g. of aluminum oxide which is washed with benzene, ether and finally acetone. The residue from evaporation of the benzene-ether fraction yields 2.54 g. of the ketone after recrystallization from hexane. An additional 240 mg. of product is obtained from the last mother liquor by sublimation of the residue at 150° under high vacuum. The total yield is 6.9 g. (53.0%).

B. Notes

1. When rigid precautions are taken to eliminate atmospheric moisture in transfers and filtrations, the deuterium content of the product is usually slightly in excess of the theoretical value.
2. The following procedures are taken from the synthesis of 3α -hydroxy-20-pregnanone described by Meystre and Miescher.¹
3. The elimination of hydrogen bromide to form the diene can be done just as well by heating the carbon tetrachloride solution for a longer time.
4. The synthesis of 3α -hydroxy-20-pregnanone can be carried out without purification of the intermediate products.
5. The 3α -hydroxy-20-pregnanone-11,12- H_2^2 prepared by Koechlin, *et al.*, according to this procedure had the following constants: m.p. $150\text{--}151^{\circ}$, $[\alpha]_D^{25} +117^{\circ}$ (in chloroform). Another crystalline modification of pregnanolone was obtained which melted at $132\text{--}133^{\circ}$; $[\alpha]_D^{25} +117^{\circ}$ (in chloroform). The infrared spectra of these two samples were identical in solution but different in suspension in mineral oil.

C. Other Preparations

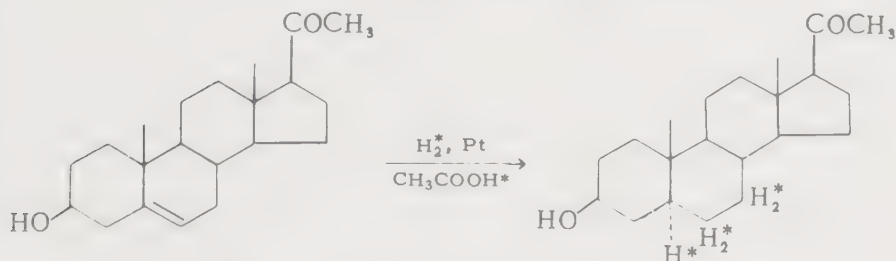
Methyl 3 α -acetoxycholanate-11,12-H₂², m.p. 135–136, has been prepared² by the catalytic hydrogenation of methyl 3 α -acetoxy-11-cholenate, essentially as described. This product contained 1.82 gram atoms of hydrogen-H² per mole.

Methyl H²-3 α -acetoxycholanate has been prepared² from methyl 3 α -acetoxy-9(11)-cholenate in the same manner. After the initial product was treated with perbenzoic acid at room temperature for 3 days, chromatographed on silica gel and recrystallized from acetone, the final product melted at 135.5–136°; $[\alpha]_D^{25} +46.3^\circ$ (acetone). This compound contained 2.26 gram atoms of hydrogen-H² per mole; the isotope should be attached at C-9, C-11 and C-12 according to the reaction mechanism proposed (see H²-3 β -cholestanol). The result with methyl 3 α -acetoxy-9(11)-cholenate, above, is also in accord with this mechanism.

¹C. Meystre and K. Miescher, *Helv. Chim. Acta*, 29, 33 (1946).

²D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, 77, 139 (1955).

3 β -ACETOXY-20-ALLOPREGNANONE-5,6,7-H₂²



D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, 77, 139 (1955).

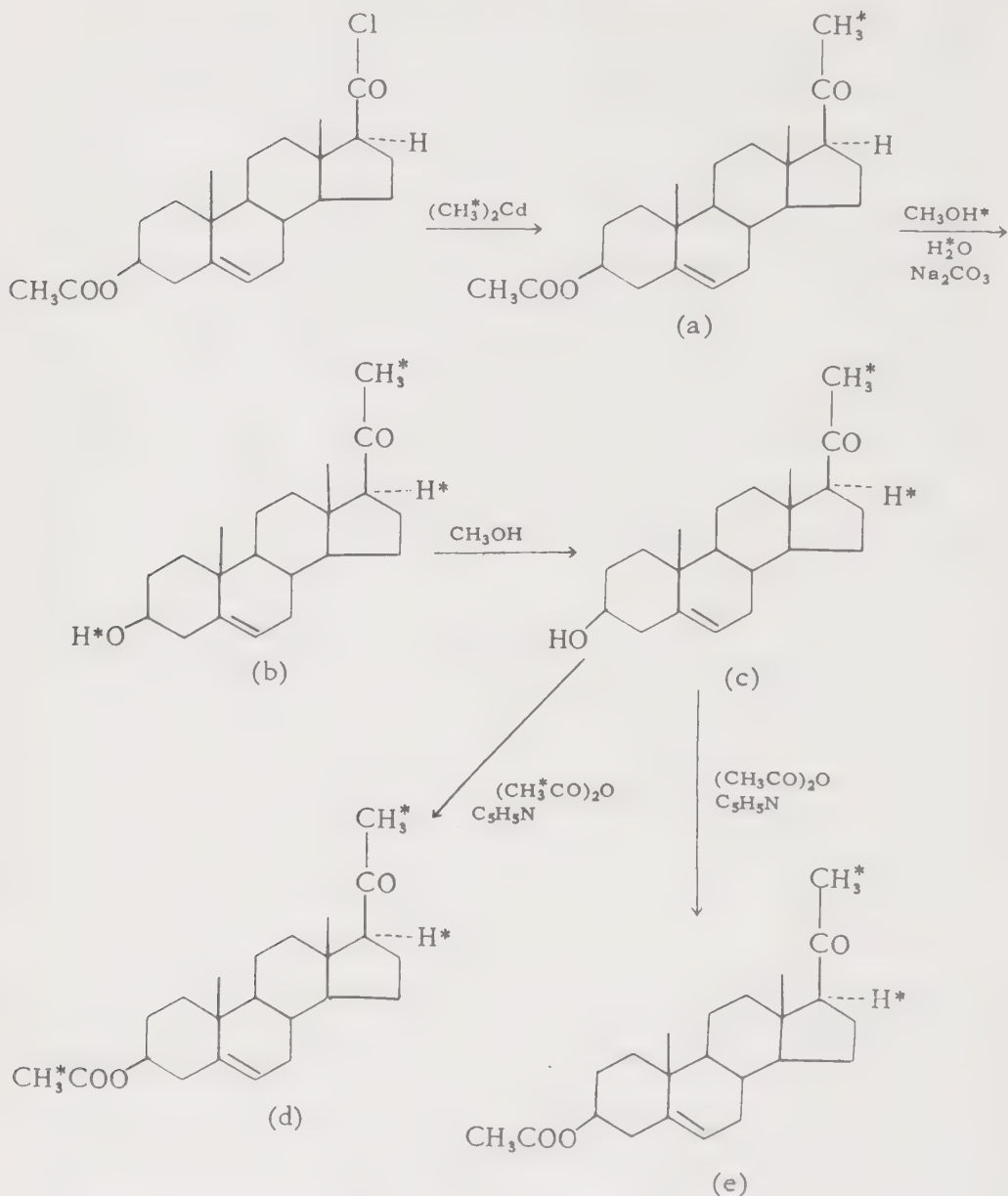
A. Procedure

A solution of 6.86 g. of 3 β -acetoxy-5-pregnen-20-one in 85 ml. of acetic acid-H² is hydrogenated with hydrogen-H₂² gas and a platinum catalyst.¹ The crude reduction product is then oxidized with 5.52 g. of *N*-bromoacetamide in a mixture of 45 ml. of *t*-butyl alcohol, 2 ml. of water and 2 ml. of pyridine. Recrystallization of the product from petroleum ether gives 3.79 g. of 3 β -acetoxy-20-allopregnanone-5,6,7-H₂², m.p. 146.5–147° (Note 1).

B. Notes

1. See Note 2, cholestane-5,6,7-H₂².

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

3 β -ACETOXY-5-PREGNEN-20-ONE-17,21- H_4^2 

B. Nolin and R. N. Jones, Can. J. Chem., 30, 727 (1952).

A. Procedure

(a) 3 β -Acetoxy-5-pregnen-20-one-21- H_3^2 . A solution of methylmagnesium- H_3^2 bromide is prepared from 0.275 g. of magnesium turnings and 0.65 ml. of methyl- H_3^2 bromide in 20 ml. of dry ether. To this solution is added 3.0 g. of cadmium bromide, and the reaction mixture is refluxed

for 2 hours with stirring. A solution of 2.14 g. of 3β -acetoxy-5-etiocolenoyl chloride in 20 ml. of benzene is added dropwise to the solution of bis(methyl- H_3^2)cadmium, and is followed by 5 ml. of benzene. The reaction mixture is refluxed for 2 hours and then cooled to room temperature. The mixture is treated with 8 ml. of water and 12 ml. of 10% hydrochloric acid, stirred for 30 minutes, and kept overnight. The product, which is extracted into ether, is washed successively with 5% sodium bisulfite, sodium hydroxide and water, and the solution is then dried over anhydrous sodium sulfate. Removal of the solvent leaves 1.25 g. of crude 3β -acetoxy-5-pregnen-20-one- $21-H_3^2$ which, after two recrystallizations from methanol and a third from methanol-acetone-water melts at $138.2-140.2^\circ$ (Note 1).

(b) 3β -Hydroxy- H^2 -5-pregnen-20-one- $17,21-H_4^2$. Crude 3β -acetoxy-5-pregnen-20-one- $21-H_3^2$, 95 mg., is dissolved in a solution of 50 mg. of anhydrous sodium carbonate in 2.0 ml. of methanol- H^2 and 0.21 ml. of water- H_2^2 . The solution is warmed on a water-bath, and a precipitate forms. The precipitate is redissolved by the addition of 2.0 ml. of methanol- H^2 , and the solution is refluxed for 2 hours. After the alcohol is distilled off, the residue is acidified with 5% aqueous hydrochloric acid, and the resultant suspension is extracted with ether. The ether solution is washed with water, dried over anhydrous sodium sulfate and evaporated to dryness (Notes 2 and 3).

(c) 3β -Hydroxy-5-pregnen-20-one- $17,21-H_4^2$. The above hydroxy- H^2 compound is treated several times with methanol and recrystallized from ether at -78° , m.p. $180.0-182.4^\circ$.

(d) 3β -Acetoxy- H_3^2 -5-pregnen-20-one- $17,21-H_4^2$. This compound is prepared from 3β -hydroxy-5-pregnen-20-one- $17,21-H_4^2$ by acetylation with bis(acetic- H_3^2) anhydride in dry pyridine. After the solution stands at room temperature overnight, the pyridine and excess anhydride are removed under vacuum.

(e) 3β -Acetoxy-5-pregnen-20-one- $17,21-H_4^2$. 3β -Hydroxy-5-pregnen-20-one- $17,21-H_4^2$ is acetylated with ordinary acetic anhydride and pyridine in the manner described above.

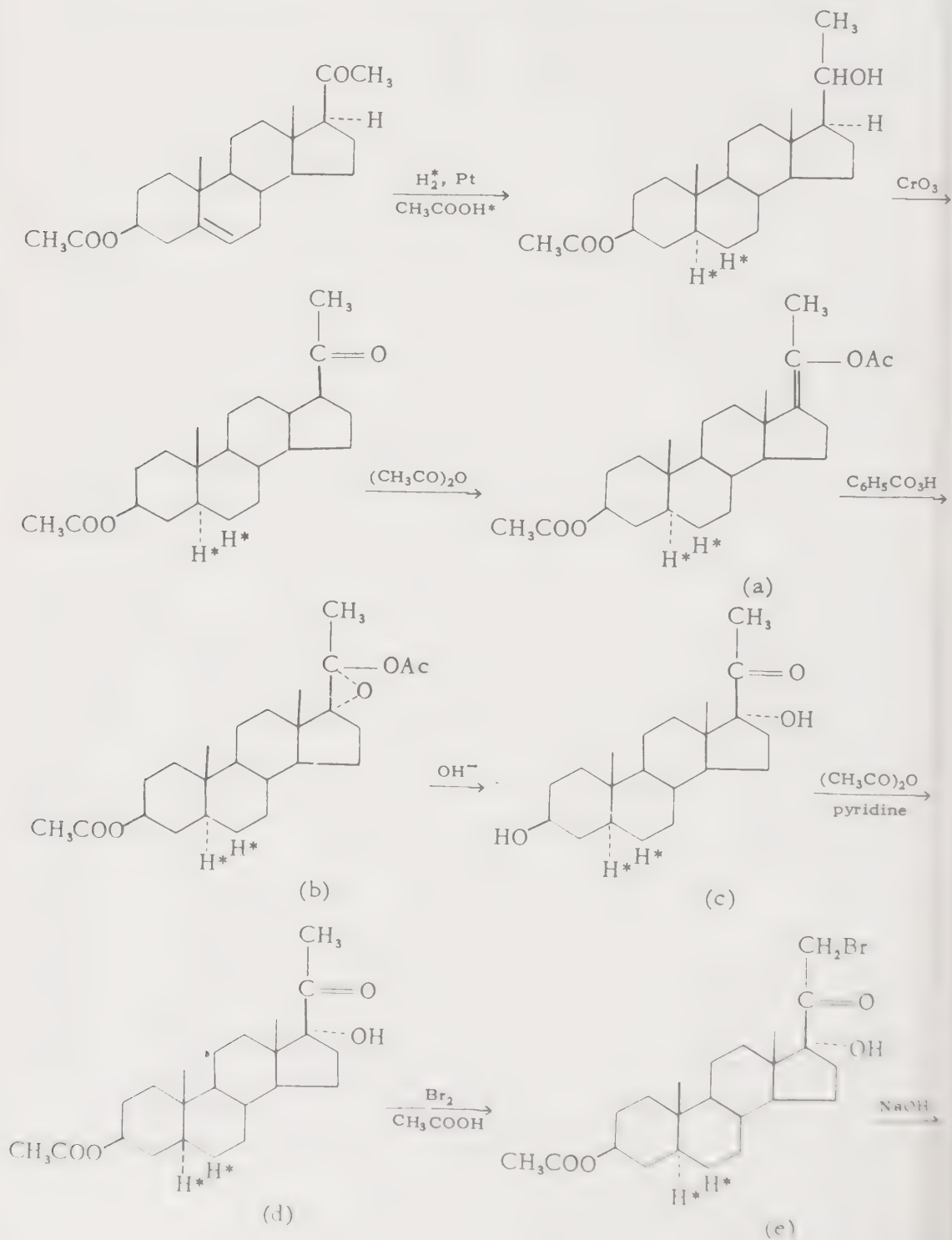
B. Notes

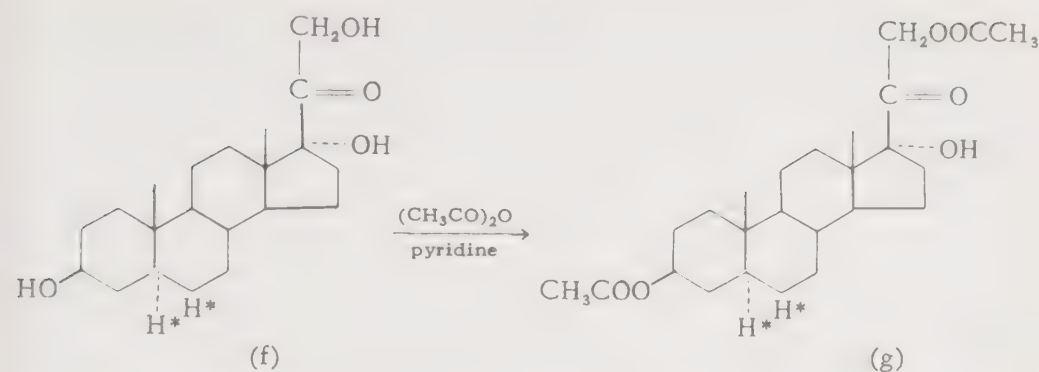
1. Although the purified product contained 2.67 deuterium atoms per molecule, when 0.77 g. of the crude ester was treated with 40 ml. of 0.1 *N* sodium hydroxide in 65% aqueous ethanol for 30 minutes, 3β -hydroxy-5-pregnen-20-one (m.p. $181.1-182.6^\circ$), containing no deuterium, was isolated.

2. By assay this compound contained 4.62 atoms of deuterium per molecule.

3. This compound was also obtained by exchange and hydrolysis of normal 3β -acetoxy-5-pregnen-20-one with a solution of anhydrous sodium carbonate in methanol- H^2 and water- H^2 refluxed overnight.

$3\beta,21$ -DIACETOXY- 17α -HYDROXY-20-ALLOPREGNANONE-5,6- H^2





T. H. Kritchevsky and T. F. Gallagher, J. Am. Chem. Soc., 73, 184 (1951).

A. Procedure (Note 1)

(a) *3β,20-Diacetoxy-17α,20-epoxy-17,20-epoxy-5,6-H₂*. *3β-Acetoxy-20-allopregnanone-5,6-H₂* (Note 2) is converted to the enol acetate according to the following procedure of Marshall.¹ A solution of 2 mmoles each of the ketone and *p*-toluenesulfonic acid in 75 ml. of acetic anhydride is distilled slowly through a short unpacked column until most of the anhydride is removed (Note 3). The residual solution is chilled, water is added, and, after a short time, the product is extracted into ether. The ether solution is washed with sodium hydroxide solution and with water and dried over sodium sulfate. After removal of the ether, the enol acetate remains as a brown amorphous residue.

(b) *3β,20-Diacetoxy-17α,20-epoxy-17,20-epoxy-5,6-H₂*. The enol acetate from 500 mg. of *3β-acetoxy-20-allopregnanone* is dissolved in 20 ml. of a 3 M solution of perbenzoic acid in benzene. After 1 hour at room temperature, the solution is diluted with ether, and the product is crystallized from petroleum ether, m.p. 173–176°. The product is recrystallized from a mixture of acetone and petroleum ether, m.p. 190–193°; $[\alpha]_D^{28} -37^\circ$ (chloroform).

(c) *3β,17α-Dihydroxy-20-allopregnanone-5,6-H₂*. The epoxy compound, above, is hydrolyzed with 200 ml. of 0.5 N sodium hydroxide in 50% alcohol at room temperature for 1 hour. The white, crystalline solid is recrystallized from methanol-acetone to obtain an 86% yield of product, m.p. 255–259°. Further recrystallization yields hexagonal plates, m.p. 257–259°; $[\alpha]_D^{25} +32^\circ$ (ethanol).

(d) *3β-Acetoxy-17α-hydroxy-20-allopregnanone-5,6-H₂*. *3β,17α-Dihydroxy-20-allopregnanone* is acetylated at room temperature with acetic anhydride and pyridine. The *3β-acetoxy* compound melts at 188–190°; $[\alpha]_D^{25} +16^\circ$ (acetone).

(e) *3β-Acetoxy-21-bromo-17α-hydroxy-20-allopregnanone-5,6-H₂*. After the addition of a small amount of dry hydrogen bromide to a solution of 100 mg. (0.266 mmole) of *3β-acetoxy-17α-hydroxy-20-allopregnanone* in 4 ml. of acetic acid, 0.318 mmole of bromine in 1.27 ml. of acetic acid is

added. After 10 minutes the solution is yellow and is completely colorless after 15 minutes. The acetic acid is removed *in vacuo* on a steam-bath. The weight of crystalline residue is 122.2 mg. After recrystallization from ethyl acetate-ether, the product melts at 194–195°; $[\alpha]_{\text{D}}^{27} + 34.5^\circ$ (chloroform).

(f) *3 β ,17 α ,21-Trihydroxy-20-allopregnanone-5,6-H₂²*. *3 β -Acetoxy-21-bromo-17 α -hydroxy-20-allopregnanone*, 201.5 mg., is dissolved in 100 ml. of 95% ethanol, and the solution is flushed with a stream of nitrogen. Then 100 ml. of 0.1 N sodium hydroxide solution is added at room temperature, and after 10 minutes the solution is acidified with dilute nitric acid. The solution is extracted with ether, and the extract is washed with small amounts of 5% sodium hydroxide and 5% sodium chloride solutions. After drying and removal of the solvent, 157 mg. of white, crystalline *3 β ,17 α ,21-trihydroxy-20-allopregnanone* is obtained.

(g) *3 β ,21-Diacetoxy-17 α -hydroxy-20-allopregnanone-5,6-H₂²*. The above trihydroxy compound is treated with acetic anhydride and pyridine overnight. After recrystallization from methanol, the diacetate melts at 206–207°; $[\alpha]_{\text{D}}^{25} + 47.6^\circ$ (chloroform) (Note 4).

B. Notes

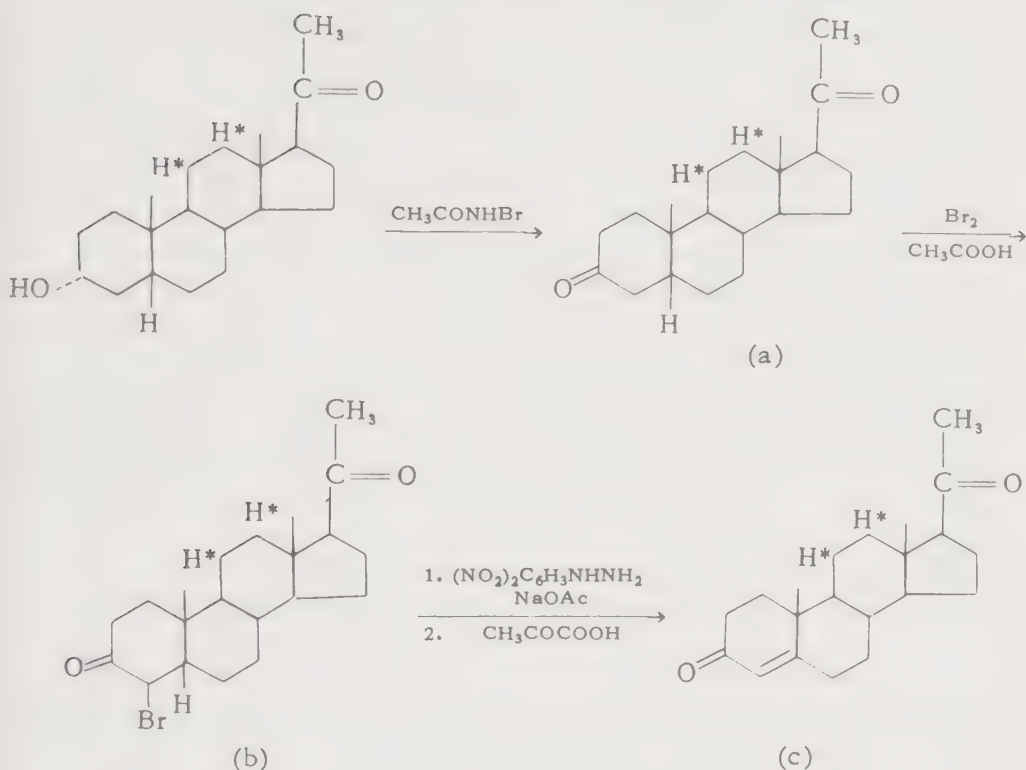
1. Although the following procedures do not include the use of deuterium, the corresponding 5,6-H₂² compounds were prepared by Kritchevsky and Gallagher.

2. *3 β -Acetoxy-20-allopregnanone-5,6-H₂²* was prepared after complete reduction of *3 β -acetoxy-5-pregnen-20-one* in acetic acid-H₂ with 99% hydrogen-H₂². The resulting *allopregnanone-3 β ,20-diol-5,6-H₂²* was oxidized with chromium trioxide to obtain the 5,6-H₂² ketone.

3. This takes 4–5 hours.

4. *3 β ,21-Diacetoxy-17 α -hydroxy-20-allopregnanone* was also prepared from *3 β -acetoxy-17 α -hydroxy-20-allopregnanone* by bromination in chloroform solution followed by alkaline hydrolysis and acetylation without isolation of intermediates. To 426 mg. of *3 β -acetoxy-17 α -hydroxy-20-allopregnanone* in 5 ml. of chloroform was added 4.45 ml. of 0.266 M solution bromine. The solution was then diluted with ethyl acetate and washed with sodium hydroxide and sodium chloride solutions. After removal of solvent, the crystalline solid was hydrolyzed with base as described above, and the reaction product was isolated and acetylated as before. After recrystallization from methanol, 252 mg. of product, m.p. 202–203° was obtained. Chromatography of the mother liquors yielded an additional 62 mg., m.p. 196–199°. The yield of the combined products, m.p. 206–207° after recrystallization from methanol, was 64%.

¹C. W. Marshall, T. H. Kritchevsky, S. Lieberman and T. F. Gallagher, J. Am. Chem. Soc., 70, 1637 (1948).

PROGESTERONE-11,12- H_2^2 

B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, *J. Biol. Chem.*, **184**, 393 (1950).

A. Procedure

(a) 3,20-Pregnanedione-11,12- H_2^2 (Note 1). To a solution of 600 mg. of 3 α -hydroxy-20-pregnanone-11,12- H_2^2 in 3 ml. of *t*-butyl alcohol is added 0.9 ml. of pyridine, 4 drops of water and 500 mg. of *N*-bromoacetamide (4 equivalents). The reaction mixture is stored overnight in a stoppered flask at 25°. To the dark solution is added ether and water, and the ether phase is washed thoroughly with dilute sodium bicarbonate, hydrochloric acid and water. The ether solution is dried over sodium sulfate and then evaporated under diminished pressure. The residue is thoroughly dried and extracted with ether (Note 2). The ether soluble material is dissolved in 1:9 benzene-petroleum ether and chromatographed on a column containing 10 times its weight of aluminum oxide. The product is eluted with benzene-petroleum ether (1:1) and crystallized from ethyl acetate-petroleum ether. The yield of 3,20-pregnanedione-11,12- H_2^2 , m.p. 120–122°, is 500 mg. (Note 3).

(b) 4-Bromo-3,20-pregnanedione-11,12- H_2^2 . A solution of 640 mg. of 3,20-pregnanedione-11,12- H_2^2 in glacial acetic acid is chilled in an ice-

bath and 4.5 ml. of a 0.49 *M* solution of bromine in glacial acetic acid (2.10 equivalents) is added dropwise within 20–30 minutes (Note 4). The acetic acid is evaporated to a few ml. at 40°, under reduced pressure, and addition of ether causes crystallization of the product. After the product is collected and recrystallized from chloroform-ether, 410 mg. (51%) of hexagonal prismatic needles is obtained; m.p. 180–184° (dec.), $[\alpha]_D +106^\circ$ (chloroform) (Notes 5 and 6).

(c) *Progesterone-11,12-H₂* (Note 7). To a solution of 100 mg. of 4-bromo-3,20-pregnanedione-11,12-H₂ and 25 mg. of sodium acetate in 3 ml. of glacial acetic acid at 60° is added 60 mg. of 2,4-dinitrophenylhydrazine (1.2 equivalents), under an atmosphere of nitrogen. The reaction mixture is kept at 60° for 20 minutes; meanwhile, the mono-2,4-dinitrophenylhydrazone of progesterone-11,12-H₂ crystallizes. To the resulting suspension, 5 ml. of chloroform, 50 mg. of sodium acetate, 3 ml. of freshly distilled pyruvic acid and 1 ml. of water are added. The resulting dark red solution is refluxed under a nitrogen atmosphere, at about 70°, until cleavage of the hydrazone is complete. This is indicated by the color change from red to yellow which is achieved within 3 hours. During this period, 3 ml. of pyruvic acid and 2 ml. of water are added in 3 portions. The solution is transferred with about 50 ml. of ether to a separatory funnel, extracted with sodium carbonate solution to remove the pyruvic acid dinitrophenylhydrazone, washed twice with 5% sodium hydroxide and several times with water, and dried over anhydrous sodium sulfate. The ether solution is evaporated to dryness, and the 78 mg. of residue is crystallized from ether-petroleum ether. The product is then sublimed at 90–100° in high vacuum and again recrystallized from ether-petroleum ether. The yield of pure progesterone-11,12-H₂, m.p. 127–129°, is 64 mg. (80%); $[\alpha]_D +200^\circ$ (chloroform), $\epsilon_{2410} = 16,800$ (methanol).

B. Notes

1. The oxidation of C-3 alcohol to keto groups in these saturated steroid compounds is readily accomplished by means of *N*-bromoacetamide.¹ The reaction proceeds smoothly and gives higher yields than the more commonly used chromium trioxide oxidation.
2. Most of the dark impurities remain undissolved.
3. Chromatography of the mother liquors afforded an additional 50 mg. of the product; the total yield was 90%.
4. The rate of addition is determined by the rate of decolorization of the bromine added.
5. From the combined mother liquors, 120 mg. (15%) of hexagonal crystals was obtained after recrystallization from chloroform-ether. This fraction melted at 175–180° and gave no depression in the melting point upon admixture with crystals from the first fraction; however, the

rotation was lower, $[\alpha]_D +83^\circ$ (chloroform). Repeated recrystallization of this fraction from chloroform-ether mixtures concentrated a product of lower rotation. After recrystallization, 25 mg. of 2,4-dibromo-3,20-pregnandione-11,12- H_2^2 , with $[\alpha]_D +70.5^\circ$ and m.p. $183-185^\circ$, was obtained. The rotation remained constant on further recrystallization.

6. Treatment of the residue from the mother liquors, in 2 ml. of glacial acetic acid, with 200 mg. of zinc dust for 1 hour at 80° affords 100 mg. (16%) of pure starting material.

7. Introduction of a 4,5-double bond in 3-oxocholane derivatives is achieved through the 4-bromo ketone as an intermediate followed by dehydrobromination. The classical dehydrobromination with pyridine or collidine² is known to give generally unsatisfactory results.³ Progesterone-11,12- H_2^2 was prepared by a modification of the new method of Mattox and Kendall,⁴ who found that the 2,4-dinitrophenylhydrazones of 4-bromo ketones of the normal steroid series are dehydrobrominated very readily and the derivatives can be cleaved in good yield to the desired α,β -unsaturated ketones. The addition of hydrobromic acid to the reaction mixture proved not only unnecessary but actually disadvantageous, since pyruvic acid was destroyed and a 20-ketone was isomerized.

C. Other Preparations

H^2 -Progesterone has been prepared⁵ by the platinum catalyzed exchange of progesterone with a 70% acetic acid- H^2 -water- H_2^2 mixture at 130° for 2 days. Chromatography of the product on alumina gave H^2 -progesterone, m.p. $120-121^\circ$, and some H^2 -17 α -progesterone, m.p. $140-142^\circ$.

¹H. Reich and T. Reichstein, *Helv. Chim. Acta*, 26, 562 (1943).

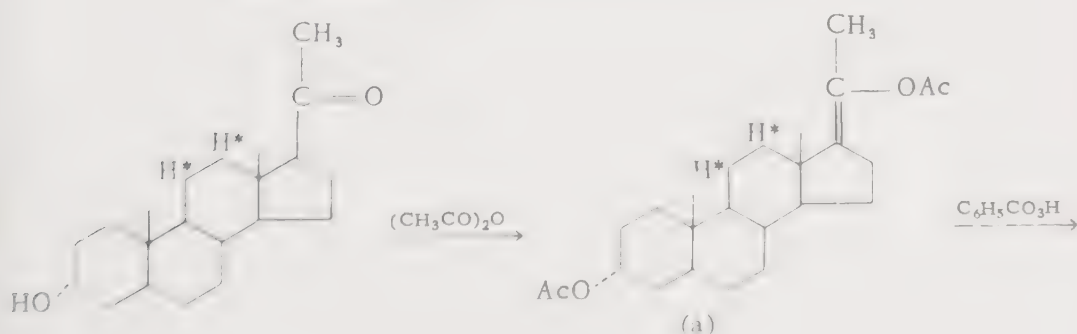
²J. Press and T. Reichstein, *ibid.*, 25, 878 (1942).

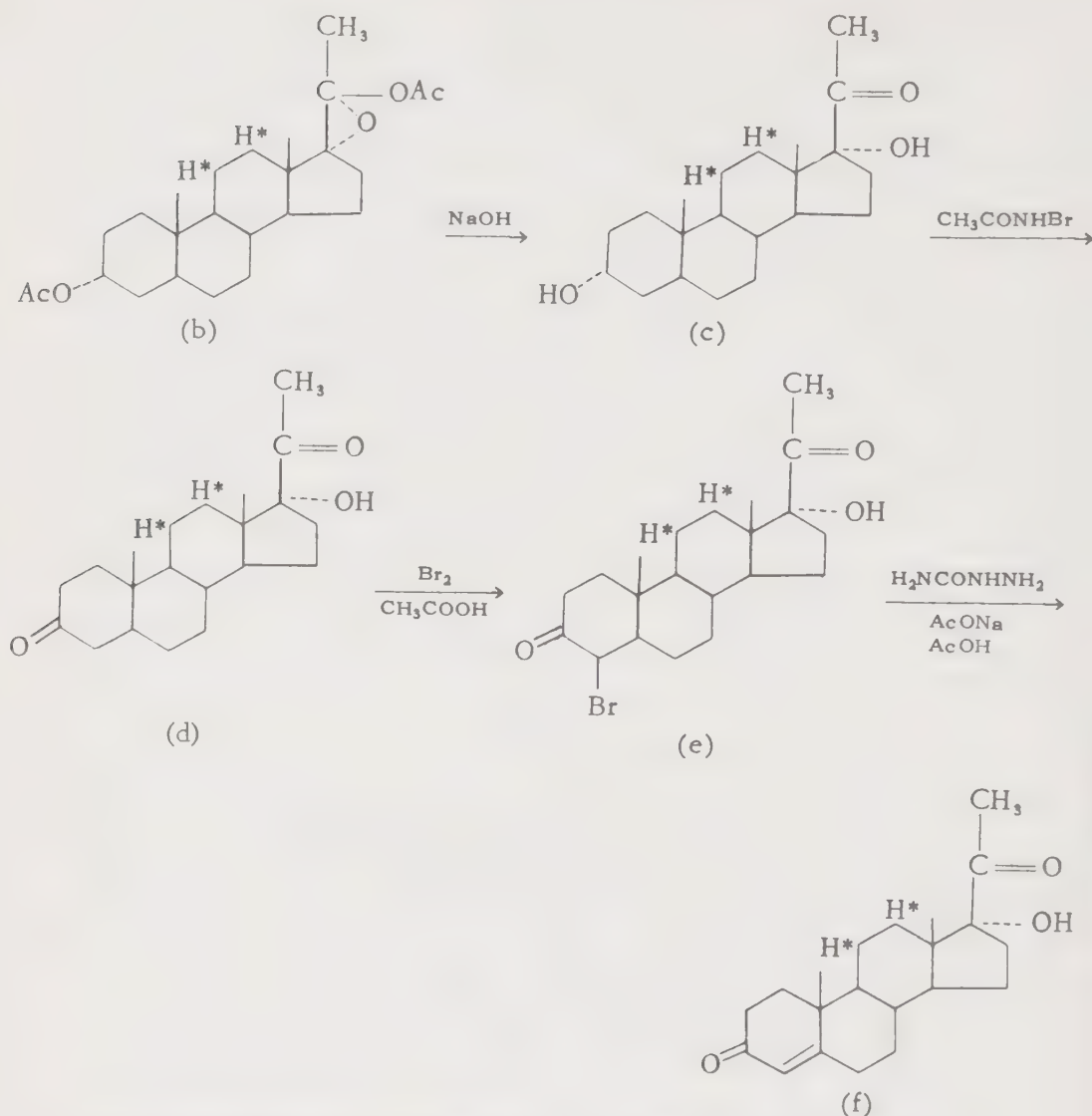
³J. von Euw and T. Reichstein, *ibid.*, 29, 654 (1946).

⁴V. R. Mattox and E. C. Kendall, *J. Am. Chem. Soc.*, 70, 882 (1948).

⁵D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, 198, 871 (1952).

17 α -HYDROXYPROGESTERONE-11,12- H_2^2





T. H. Kritchevsky and T. F. Gallagher, J. Am. Chem. Soc., 73, 184 (1951).

A. Procedure (Note 1)

(a) 3 α ,20-Diacetoxy-17 α ,20-epoxy-17-pregnene-11,12- H^2 . 3 α -Hydroxy-20-pregnanone 11,12- H^2 is converted to its enol acetate according to the procedure of Marshall;¹ see 3 β ,20-diacetoxy-17 α -allopregnene-5,6- H^2 . After chromatography of the reaction mixture, the colorless syrupy product is obtained in benzene solution.

(b) 3 α ,20-Diacetoxy-17 α ,20-epoxy-17-pregnene-11,12- H^2 . The above benzene solution of enol acetate is treated with 50 ml. of 3.5 M perbenzoic acid in benzene. The temperature increases spontaneously, and cooling is necessary. After 1 hour, ethyl acetate is added, and the solution is extracted with 5% sodium hydroxide and with water. After removal of the

solvent, the product is crystallized from petroleum ether and recrystallized from acetone-petroleum ether, m.p. 167-168°; $[\alpha]_D^{25} -6.3^\circ$ (chloroform) (Note 2).

(c) *3 α , 17 α -Dihydroxy-20-pregnanone-11,12- H_2^2* . The preceding *3 α , 20-diacetoxy-17 α , 20-epoxypregnane-11,12- H_2^2* is saponified with 500 ml. of 0.3 *N* sodium hydroxide in 50% ethanol at room temperature for 1 hour. The crude product, 5.03 g., is crystallized from acetone to obtain 4.05 g. of *3 α , 17 α -dihydroxy-20-pregnanone-11,12- H_2^2* , m.p. 208-210° (Note 3). The total yield, including product isolated as the acetate (Note 4), is 79% (Note 5).

(d) *17 α -Hydroxy-3, 20-pregnanedione-11,12- H_2^2* . To a solution of 100 mg. (0.299 mmole) of *3 α , 17 α -dihydroxy-20-pregnanone-11,12- H_2^2* in 2 ml. of *t*-butyl alcohol is added 82.5 mg. (0.598 mmole) of *N*-bromoacetamide, 0.1 ml. of water and 0.15 ml. of pyridine. After 18 hours at room temperature, the color of the reaction mixture turns from light yellow to orange (Note 6). The reaction mixture is extracted with acidic, basic and sodium chloride solutions. The product, m.p. 203-207°, weighs 96 mg. Upon recrystallization from acetone, the product forms irregular prisms which melt at 215-217°; $[\alpha]_D^{25} +53.9^\circ$ (ethanol).

(e) *4-Bromo-17 α -hydroxy-3, 20-pregnanedione-11,12- H_2^2* . *17 α -Hydroxy-3, 20-pregnanedione-11,12- H_2^2* , 370.6 mg. (1.108 mmoles), is dissolved in 12 ml. of acetic acid and, with agitation and cooling, 0.5 ml. of 0.2457 *M* solution of bromine in acetic acid is added dropwise. When the bromine color disappears, dropwise addition is continued with 4.12 ml. of an acetic acid solution which is 0.2526 *M* with respect to bromine and 0.25 *M* with respect to sodium acetate. The reaction mixture is then diluted with ethyl acetate and washed with sodium hydroxide and sodium chloride solutions. Removal of solvent leaves a white crystalline product. After two recrystallizations from chloroform-ether, the product, m.p. 186-187° (dec.), weighs 328 mg. (72%); $[\alpha]_D^{25.5} +34.0^\circ$ (chloroform) (Note 7).

(f) *17 α -Hydroxyprogesterone-11, 12- H_2^2* . A mixture of 133 mg. of *4-bromo-17 α -hydroxy-3, 20-pregnanedione-11,12- H_2^2* in 27.5 ml. of acetic acid, 110 mg. of sodium acetate, 108 mg. of semicarbazide hydrochloride and a few drops of water is heated at 67° for two hours in a nitrogen atmosphere (Note 8). Then, 2 ml. of water, 1 ml. of redistilled pyruvic acid and 300 mg. of sodium acetate are added, and heating is continued for two hours. After the mixture is cooled, 200 ml. of water is added, and a precipitate of fine needles forms. The crude product, 49 mg., is collected and recrystallized from acetone to obtain 22 mg. of product, m.p. 209-212°; $\epsilon_{2420} = 16,400$ (ethanol). The combined mother liquor and aqueous filtrate are extracted with ethyl acetate, which is then washed with dilute sodium hydroxide and with water. A residue of 90 mg. is obtained by removal of the solvent. After recrystallization from acetone,

the yield of product, m.p. 207–210°, is 63 mg. Recrystallization of the combined crystalline products from acetone affords an 80% yield of 17 α -hydroxyprogesterone-11,12-H₂², m.p. 218–219°; ϵ_{2420} = 16,500 (ethanol) (Note 9).

B. Notes

1. The procedures described were those used by Kritchevsky and Gallagher for preparing the corresponding unlabeled compounds.

2. The product did not exhibit a hydroxyl band in the infrared spectrum.

3. After further purification, an analytically pure sample melted at 213–214°, $[\alpha]_D^{25} + 63^\circ$ (ethanol), and the infrared spectrum was identical with that of an authentic sample.

4. The residue (0.99 g.) from the crystalline dihydroxy compound was acetylated and chromatographed to obtain an additional 360 mg. of 3 α -acetoxy-17 α -hydroxy-20-pregnanone-11,12-H₂², m.p. 198–199.5°.

5. Chromium trioxide oxidation of the enol acetate gave 3 α ,17 α -dihydroxy-20-pregnanone, which was converted to 3 α -acetoxy-17 α -hydroxy-20-pregnanone. The yield based on enol acetate was 13.6%.

6. As indicated by iodometric titration, 117% of the stoichiometric amount of bromine was consumed in the reaction.

7. The crystalline residues were more levorotatory and were reduced with zinc in acetic acid at room temperature to recover the starting compound.

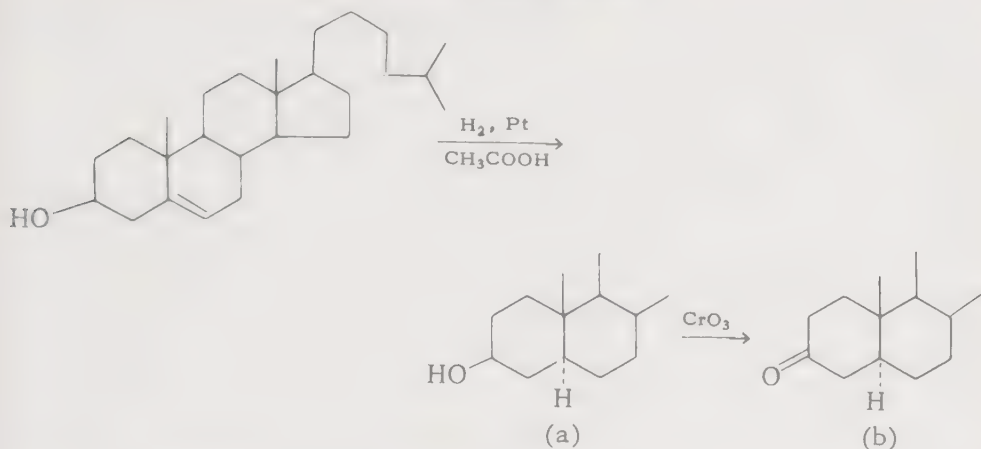
8. This dehydrobromination is a modification² of the procedure of Mattox and Kendall.³

9. 4-Bromo-17 α -hydroxy-3,20-pregnanedione was also dehydrobrominated in pyridine at 110–130° under an atmosphere of nitrogen (sealed tube).

¹C. W. Marshall, T. H. Kritchevsky, S. Lieberman and T. F. Gallagher, J. Am. Chem. Soc., 70, 1637 (1948).

²B. A. Koechlin, T. H. Kritchevsky and T. F. Gallagher, J. Biol. Chem., 184, 393 (1950).

³V. R. Mattox and E. C. Kendall, J. Am. Chem. Soc., 70, 882 (1948).

H²-3-CHOLESTANONE

D. K. Fukushima and T. F. Gallagher, J. Biol. Chem., 198, 861 (1952).

A. Procedure (Note 1)

(a) *H²-3 β -Cholesterol* (Note 2). To a solution of 900 mg. of H²-cholesterol in 75 ml. of acetic acid is added 250 mg. of platinum oxide catalyst.¹ The reaction mixture is shaken with hydrogen at room temperature until the calculated amount of hydrogen is consumed. The reduced product is refluxed with 3% methanolic potassium hydroxide for 30 minutes. After recrystallization from acetone the product melts at 142–144°, (Note 3).

(b) *H²-3-Cholestanone*. The above H²-3 β -cholestanol is oxidized with a solution of chromic oxide in acetic acid for 4 hours at room temperature. The product is chromatographed on silica gel, and the eluates from ether-petroleum ether (1:9) are combined and recrystallized from methanol. The product melts at 128.5–129.5° (Note 4).

B. Notes

1. The following is part of a degradative study to determine the location of the isotope in H²-cholesterol prepared by catalytic exchange.

2. According to this study, the platinum-catalyzed exchange reaction with cholesterol in 70% solution of acetic-H₂ acid-H² in water-H₂ yields H²-cholesterol with 40% of the incorporated isotope at C-6, about 3% at C-3 and 52% at or among C-24, C-25, C-26 and C-27. See H²-cholesterol, Other Preparations.

3. According to the deuterium analysis of the product, almost half of the cholesterol deuterium was exchanged with hydrogen during the catalytic reduction. There was no loss of deuterium, in a similar experiment, when hydrogen was omitted from the medium.

4. The deuterium content was unchanged after the H²-3-cholestanone was refluxed for 2 hours with 2.5% methanolic potassium hydroxide. This was taken to indicate no isotope at C-2 or C-4.

C. Other Preparations

3 β -Cholesterol-5,6,7- H_3^2 , m.p. 141–142°, has been prepared² by the hydrolysis of 3 β -cholestanyl-5,6,7- H_3^2 acetate, which was obtained by the hydrogenation of 5-cholesten-3 β -yl acetate with hydrogen- H_2^2 and a platinum catalyst. H^2 -3 β -Cholesterol has been obtained³ by platinum catalyzed exchange with water- H_2^2 and acetic acid.

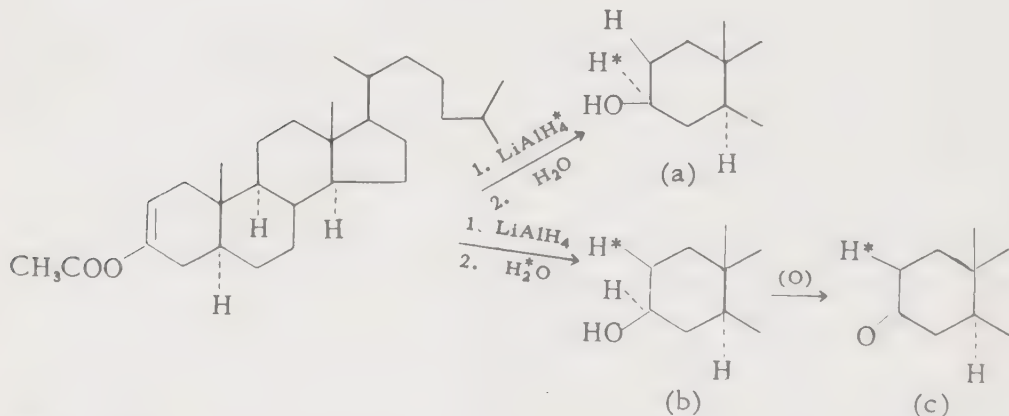
3-Cholestanone-5,6,7- H_3^2 , m.p. 120.5–130°, has been prepared² by oxidation of 3 β -cholestanol-5,6,7- H_3^2 with acidic dichromate solution.⁴ In these compounds, it was shown that the isotope was located at C-5, C-6 and C-7. A mechanism of hydrogenation was proposed to explain the distribution found. H^2 -3-Cholestanone was isolated³ as a by-product in the preparation of H^2 -cholesterol by exchange.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

²D. K. Fukushima and T. F. Gallagher, *J. Am. Chem. Soc.*, 77, 139 (1955).

³K. Bloch and D. Rittenberg, *J. Biol. Chem.*, 149, 505 (1943); H. S. Anker and K. Bloch, *J. Am. Chem. Soc.*, 66, 1762 (1944).

⁴*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 139.

3-CHOLESTANONE-2- H_3^2 

W. G. Dauben and J. F. Eastham, *J. Am. Chem. Soc.*, 75, 1718 (1953).

A. Procedure (Note 1)

(a) 3 β -Cholesterol-3 α - H^2 . The reduction of 2-cholesten-3 β -yl acetate with lithium aluminum hydride- H_4^2 is according to the procedure described by Dauben¹ (Note 2). To 0.9 g. of 2-cholesten-3 β -yl acetate in 25 ml. of anhydrous ether is added a slurry of 0.30 g. of lithium aluminum hydride- H_4^2 in 25 ml. of ether, under a nitrogen atmosphere. The reduction complex and excess hydride- H_4^2 are decomposed with 30 ml. of cold 10% sulfuric acid. More ether is added to the organic phase, and the ethereal solution is washed successively with dilute sulfuric acid, saturated aqueous sodium bicarbonate solution and water. The solution is dried

and, after evaporation of the solvent, the residue is dissolved in hexane and chromatographed on alumina (Note 3). Elution with hexane, 15% ether in hexane (by volume) and 25% ether in hexane, in this order, gives, respectively, 161 mg. (20%) of 3-cholestanone, m.p. 129–130°, $[\alpha]_D^{25} + 41.3^\circ$; 130 mg. (16%) of 3 α -cholestanol-3 β -H², m.p. 180–182°, $[\alpha]_D^{25} + 24^\circ$; and 456 mg. (56%) of 3 β -cholestanol-3 α -H², m.p. 141.5–142.5°, $[\alpha]_D^{25} + 22.7^\circ$ (Note 4).

(b) 3 β -Cholestanol-2-H². The reduction of 0.9 g. of 2-cholesten-3 β -yl acetate with 4.2 mmoles of lithium aluminum hydride is conducted as described above. After the solution is stirred for 1 hour, 0.5 g. (25 mmoles) of water-H₂ is added, and the mixture is stored under a nitrogen atmosphere for 10 hours. Additional anhydrous ether is added, and the mixture is stirred for 2 hours and is then centrifuged. The ether is decanted, and the residue is extracted twice with additional dry ether. The combined ether solution is filtered and evaporated to obtain 685 mg. of solid product. This crude product is dissolved in hexane and chromatographed on alumina (Note 5). Elution of the products, as described above, gives 100 mg. of 3 α -cholestanol-2-H₁², m.p. 180–182°, and 286 mg. of 3 β -cholestanol-2-H₁², m.p. 140–142°. After recrystallization of the latter, the m.p. is 141.5–142.5°, $[\alpha]_D^{25} + 23^\circ$.

(c) 3-Cholestanone-2-H₁². A solution of 300 mg. of cholestanol-2-H₁² in 6 ml. of dry benzene is cooled in an ice-bath until the benzene solidifies. A solution containing 0.5 g. of crystalline sodium dichromate, 0.4 ml. of glacial acetic acid, 0.7 ml. of concentrated sulfuric acid and 2 ml. of water is cooled to 0°. This solution is added in one portion to the frozen benzene solution, and the flask is flushed with nitrogen and then stoppered. With magnetic stirring, the reaction mixture is allowed to warm slowly to 15°; the mixture is then stirred for 10 hours. The benzene layer is separated and washed with water and 10% sodium bicarbonate solution (Note 6). After the solution is dried over sodium sulfate, the solvent is removed; final traces of benzene are removed by azeotropic distillation with ethanol. The product is crystallized directly from this latter solvent. The yield of 3-cholestanone-2-H₁², m.p. 129.5–130.0°, $[\alpha]_D^{25} + 41.5^\circ$, is 245 mg. (82%) (Note 7).

B. Notes

1. The mechanism of the reduction of steroid enol acetates is discussed by Dauben and Eastham.

2. The preparation of 2-cholesten-3-yl acetate, (3-cholestanone enol acetate), has been described.¹

3. The adsorbent employed was Merck and Co., Inc., Reagent Aluminum Oxide. Approximately 25 g. of alumina was used per gram of steroid.

4. The infrared spectra of the two sterols showed the characteristic C-H² absorption at 2060 cm.⁻¹, and this band was absent in the ketone.

5. An alumina, which had been neutralized and activated,² was first used. This adsorbent was too active, and addition of a few per cent of methanol to the ether-hexane solvent was necessary in order to elute the sterols. A second chromatograph on less active alumina (Note 3) was necessary to effect separation of the products.

6. Both were precooled in an ice-bath.

7. The product retained the C-H² infrared absorption band at 2060 cm. and a deuterium content equivalent to that of the 3 β -cholestanol-2-H².

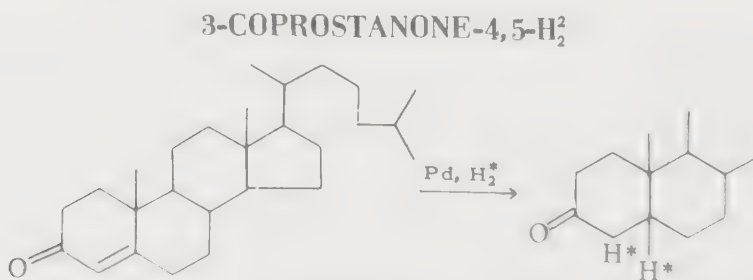
C. Other Preparations

A method has been described³ for the location of deuterium atoms attached to cyclohexane rings. The data indicate that infrared analysis can be used to differentiate between the axial and equatorial orientations of deuterium in cyclohexane systems. The reported studies were carried out with the following steroids, which were labeled at known positions by means of reactions of known and specific stereochemical course. Cholestane-3 α -H² was prepared by the reduction of 3 β -cholestanyl *p*-toluenesulfonate with lithium aluminum hydride-H². 3,5-Cyclocholestane-6 β -H² was prepared by the reduction of cholesteryl *p*-toluenesulfonate with hydrogen-H², and 7-hydroxy-3 β -cholestanyl-6 β -H² acetate by hydrogenation of 6 α ,7 α -epoxy-3 β -cholestanyl acetate with hydrogen-H². 3 β -Acetoxy-7-cholestanone-6 β -H² was prepared by oxidation of the last named product above. Cholestane-3 β -H² was obtained by treatment of 3 β -cholestanyl-3 α -H² *p*-toluenesulfonate with lithium aluminum hydride. 5-Cholestene-3 β -H² was prepared by hydrogenation of cholesteryl *p*-toluenesulfonate with hydrogen-H².

¹W. G. Dauben, R. A. Micheli and J. F. Eastham, J. Am. Chem. Soc., 74, 3852 (1952).

²C. W. Shoppee and G. H. R. Summers, J. Chem. Soc., 1950, 687.

³E. J. Corey, R. A. Sneen, M. G. Danaher, R. L. Young and R. L. Rutledge, Chem. & Ind. (London), 1954, 1294.



R. Schoenheimer, D. Rittenberg and M. Graff, J. Biol. Chem., 111, 183 (1935).

A. Procedure

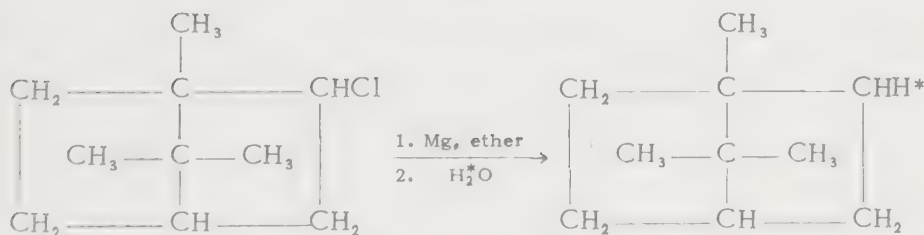
4-Cholesten-3-one,¹ 10 g., in 50 ml. of dry ether is shaken with 0.5 g. of active palladium in an atmosphere of hydrogen- H_2^2 . After one mole of hydrogen- H_2^2 per mole of cholestenone is absorbed, the reaction stops. The catalyst is collected and washed with ether. The total filtrate is concentrated to dryness, and the residue is recrystallized from aqueous alcohol (Note 1). To remove the by-product, 3β -coprostanol-3,4,5- H_3^2 , the entire product is dissolved in alcohol, and 50 ml. of 1% digitonin in alcohol is added. The alcoholic solution is concentrated to dryness, and the residue is extracted with ether. After removal of ether and recrystallization of the residue from aqueous alcohol the product melts at 62° , $[\alpha]_D^{25} + 36.9^\circ$ (chloroform) (Note 2).

B. Notes

1. A sample of the product gave a small amount of precipitate with digitonin indicating that some coprosterol had been formed by reduction of the carbonyl group. This by-product could not be removed by recrystallization.

2. The product gave no precipitate with digitonin and the melting point of an admixture with coprostanone, prepared with ordinary hydrogen, (m.p. 62° , $[\alpha]_D^{23} + 36.3^\circ$) showed no depression.

¹R. Schoenheimer, J. Biol. Chem., 110, 461 (1935).

CAMPHANE-2- H_1^2 

E. Biilmann, K. A. Jensen and E. Knuth, Ber., 69, 1031 (1936).

A. Procedure (Note 1)

To 3.75 g. of magnesium and 20 ml. of dry ether, in a flask equipped with stirrer, reflux condenser and drying tube, is added 1 g. of ethyl bromide with stirring, and then, with stirring and heating, a solution of 25 g. of 2-bornyl chloride in 20 ml. of ether is added dropwise. After the mixture is stirred two hours longer, it is cooled in ice and, with stirring, 5 ml. of water- H_2^2 is added dropwise (Note 2). After 0.5 hour of stirring

at room temperature, the reaction mixture is filtered and dried over potassium carbonate. Most of the ether is removed on a water-bath, and the residue is distilled through a 30-cm. Vigreux column. The fraction which boils at 156–157° weighs 8.2 g. and melts at 125°. After two recrystallizations, the product, m.p. 153.5–154°, has a rotation, $\alpha = 0.07^\circ$ (Note 3).

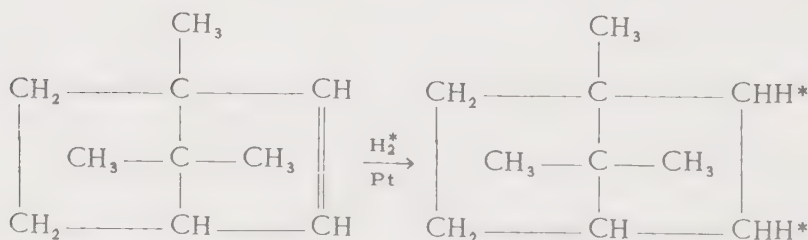
B. Notes

1. The entire reaction was carried out in a hydrogen atmosphere.
2. The amount of water- H_2^2 used was twice the calculated quantity.¹
3. The optical activity of the camphane-2- H_1^2 was completely removed by shaking the product in ether with platinized carbon in a hydrogen atmosphere. In later work Biilmann, Jensen and Bak² obtained D-rotatory products from the Grignard reagent with both water and water- H_2^2 . Both products were still D-rotatory after several recrystallizations, hence still impure, although they had the highest melting points recorded in the literature for camphane. Both products were made optically inactive by the treatment with platinized charcoal and hydrogen.

¹A. Hesse, Ber., 39, 1127 (1906).

²E. Biilmann, K. A. Jensen and B. Bak, Ber., 69B, 1947 (1936).

CAMPHANE-2,3- H_2^2



M. T. Leffler and R. Adams, J. Am. Chem. Soc., 58, 1555 (1936).

A. Procedure

The apparatus employed for the reduction of bornylene with hydrogen- H_2^2 is the same as that previously described¹ (see succinic-2,3- H_2^2 anhydride). Two reduction tubes are used, the charge in each tube being 1.36 g. of L-bornylene (Note 1), 0.05 g. of platinum catalyst and 8 ml. of ethyl acetate (Note 2). After the mixture is shaken with hydrogen- H_2^2 (~100%) for two hours, the reduction is complete. In order to precipitate the crude camphane-2,3- H_2^2 , the filtrate from removal of the catalyst is shaken with one l. of cold water. The crude product is collected, dried for a short time over phosphorus pentoxide and finally sublimed at room temperature from metallic sodium (Note 3); m.p. 143° (Note 4); d_4^{152}

0.7552; $[\alpha]_D^{25} + 0.02 \pm 0.01^\circ$ (c, 0.85 g. in 5 ml. of ethyl acetate, $l = 1$) (Note 5).

B. Notes

1. D-Borneol was converted into D-methyl bornyl xanthate, according to Tschugaeff,² which was purified by recrystallization from alcohol, m.p. 57–58°, and converted to L-bornylene by dry distillation at 170–190°. The crude bornylene was repeatedly sublimed from metallic sodium to obtain a sample free of sulfur impurities; m.p. 105–106.5°; $[\alpha]_D^{25} - 10.66^\circ$ (c, 4.627 in toluene), $[\alpha]_D^{25} - 9.57^\circ$ (c, 16.00 in ethyl acetate); d_4^{31} 0.7666.

2. The solvent was dry and acid and alcohol-free.

3. The camphane obtained in this manner gave no test for unsaturation with bromine in glacial acetic acid.

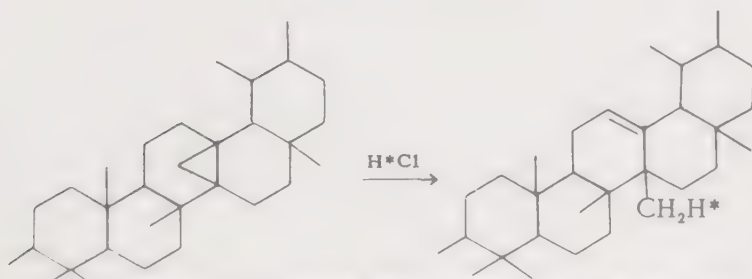
4. The low melting point for camphane indicates the impurity, tricycylene, carried through from bornylene.

5. The rotation of the corresponding hydrogen-reduced camphane was identical, due to some impurity.

¹A. McLean and R. Adams, J. Am. Chem. Soc., 58, 804 (1936).

²L. Tschugaeff, Ber., 32, 3332 (1889); Ann., 388, 280 (1912).

α -AMYRENE-27- H_1^2



D. H. R. Barton, J. E. Page and E. W. Warnhoff, J. Chem. Soc., 1954, 2715.

A. Procedure

A solution of phyllanthane (Note 1) in alcohol-free chloroform is treated with hydrogen- H^2 chloride according to the procedure of Barton and de Mayo;¹ see 9(11)-lanostene-19- H_1^2 . The product is α -amyrene-27- H_1^2 , m.p. 118–119°, $[\alpha]_D + 89^\circ$ (c, 1.65 in chloroform) (Note 2).

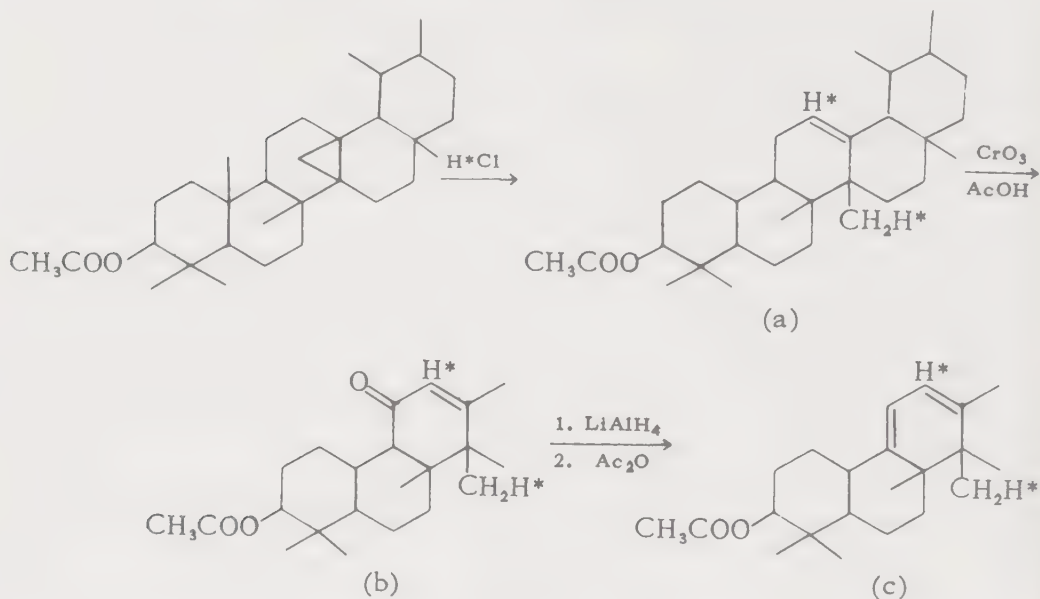
B. Notes

1. Phyllanthane, m.p. 173–174°, $[\alpha]_D + 43^\circ$ (c, 1.91 in chloroform), was prepared from phyllanthol according to the procedure of Barton and de Mayo.¹

2. An authentic sample of α -amyrene had m.p. 117–118° and $[\alpha]_D +93^\circ$ (c, 2.08 in chloroform).

¹D. H. R. Barton and P. de Mayo, J. Chem. Soc., 1953, 2178.

α -AMYRA-10,12-DIEN-2-YL-12-H²-27-H₁² ACETATE



D. H. R. Barton and P. de Mayo, J. Chem. Soc., 1953, 2178.

A. Procedure

(a) α -Amyren-2-yl-12-H²-27-H₁² Acetate (Note 1). A solution of 150 mg. of phyllanthyl acetate in 5 ml. of dry alcohol-free chloroform, containing 2 drops of water-H₂², is stirred magnetically, and the flask is evacuated. Hydrogen-H² chloride is admitted to the flask, which is then re-evacuated and again filled with the gas. The flask is sealed and, after the solution is stirred for 15 minutes, it is left overnight at room temperature. After the solvent is removed *in vacuo*, the residue is recrystallized from chloroform-methanol; yield 115 mg.

(b) 2-Acetoxy-11- α -amyrene-12-H²-27-H₁². To a solution of 55.2 mg. of α -amyren-2-yl-12-H²-27-H₁² acetate in 4 ml. of acetic acid at 85° is added 28 mg. of chromium trioxide in 1 ml. of acetic acid during 45 minutes. Heating of the mixture is then continued for 30 minutes (Note 2). The product is crystallized from chloroform-methanol; the yield of 2-acetoxy-11- α -amyrene-12-H²-27-H₁², m.p. 270–272°, is 26 mg.

(c) α -Amyra-10,12-dien-2-yl-12-H²-27-H₁² Acetate. A mixture of 300 mg. of 2-acetoxy-11- α -amyrene-12-H²-27-H₁² in 80 ml. of dry ether and 300 mg. of lithium aluminum hydride in 10 ml. of dry ether is refluxed for 30 minutes. The crude product is then heated under reflux for 20 minutes in a

solution of 20 mg. of *p*-toluenesulfonic acid in 11 ml. of acetic anhydride. The crude product is crystallized from methanol, m.p. 166–167°, $[\alpha]_D^{+335}$ (c, 2.15 in chloroform).

B. Notes

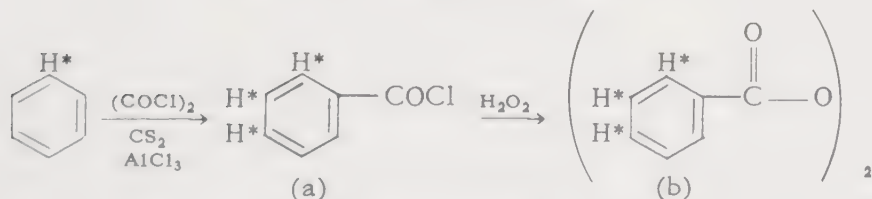
1. Barton and de Mayo did not have sufficient experimental data, at that time, to definitely assign the location of the deuterium introduced into these compounds. However, it appears quite likely, in view of the later work of Barton, Page and Warnhoff,¹ that the names given and the structures represented do accurately describe these compounds; see lanostane-19- H_1^2 and α -amyrene-27- H_1^2 . The location of 1 atom of deuterium at C-12 is somewhat in doubt however. It was found that the treatment of phyllanthyl acetate with hydrogen- H^2 chloride introduced 2.65 atom per cent excess deuterium instead of the expected 1.9 atom per cent. Since hydrolysis of the ester and reacetylation did not change the deuterium content, the possibility that the acetate methyl group contained deuterium was eliminated. A mechanism for the replacement of the vinyl hydrogen at C-12 by deuterium was suggested by Fukushima.²

2. Some of the oxidizing agent remains unchanged.

¹D. H. R. Barton, J. E. Page and E. W. Warnhoff, J. Chem. Soc., 1954, 2715.

²Communication from D. K. Fukushima to Barton and de Mayo.

BIS(BENZOYL- $H_{1/3}^2$) PEROXIDE



T. Yosida, Bull. Chem. Soc. Japan, 23, 209 (1950).

A. Procedure

(a) *Benzoyl- $H_{1/3}^2$ Chloride* (Note 1). Benzoyl- $H_{1/3}^2$ chloride is prepared according to the following method of Staudinger.¹ A solution of 12.7 g. of oxalyl chloride in carbon disulfide is dropped slowly, with good cooling, into a mixture of 25 ml. of benzene and 26 g. of aluminum chloride in 50 ml. of carbon disulfide. After removal of the solvent and unreacted reagents, a nearly quantitative yield of benzoyl chloride is obtained by distillation.

(b) *Bis(benzoyl- $H_{1/3}^2$) Peroxide*. Benzoyl peroxide is prepared from benzoyl chloride and an alkaline solution of hydrogen peroxide according to Pechmann and Vanino² or with sodium peroxide as reported by Gambar-

B. Notes

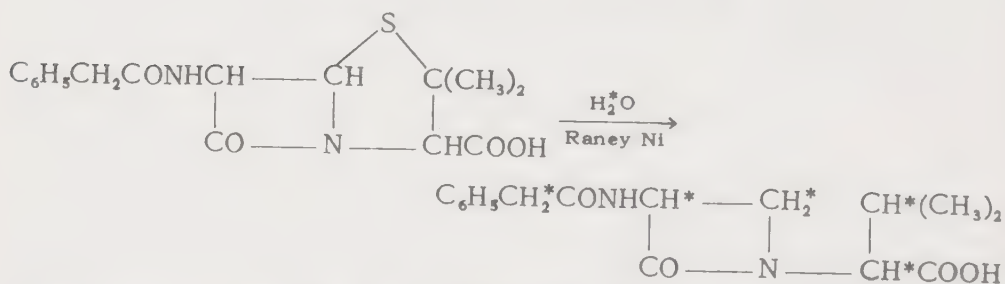
2. Ice may be added to the reaction mixture.

¹H. Staudinger, Ber., 41, 3558 (1908).

²H. Pechmann and L. Vanino, *ibid.* 27, 1511 (1894).

³S. Gambarjan, *ibid.* 42, 4004 (1909).

H²-DETHIOBENZYL PENICILLIN



E. Kaczka and K. Folkers, *The Chemistry of Penicillin*, Princeton University Press, Princeton, New Jersey, 1949, p. 243.

A. Procedure

Sodium benzylpenicillin (penicillin-G), 200 mg., is heated under reflux for 15 minutes with about 2 g. of "deuterized" Raney nickel (Note 1) in about 10 ml. of 99.5% water-H₂. After the mixture is cooled, the nickel, removed by filtration, is washed 6 times with 0.5-ml. portions of water. The filtrate and washings are acidified with one equivalent of hydrochloric acid, and the precipitated aluminum hydroxide is separated from the solution. The filtrate, concentrated to about 10 ml. *in vacuo*, is seeded with crystals of dethiobenzylpenicillin and cooled in an ice-bath. After one hour, the crystalline product is collected and air-dried. The yield of material melting at 91-95° is 81 mg. (47%). The product is purified by dissolution in 2 ml. of 95% ethanol, to which then is added 15 ml. of water.

Crystals, which form in rosettes of prismatic needles, are separated by centrifugation, washed with cold water and dried *in vacuo* over phosphorus pentoxide. The yield of the first crop of crystallized material is 40 mg., micro m.p. 104–106° (Note 2).

B. Notes

1. The catalyst was prepared according to the method described in *Organic Syntheses*¹ as modified by Mozingo, *et al.*²

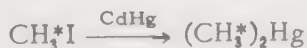
2. Deuterium analysis on the recrystallized material indicated that the dethiobenzylpenicillin contained 6.5 stable deuterium atoms per molecule. Hydrogenolysis of sodium benzylpenicillin was also effected smoothly at room temperature, giving dethiobenzylpenicillin containing 5.8 atoms of deuterium per molecule. From the same reaction mixture was isolated a small amount of phenylacetyl-L-alanyl-D-valine, m.p. 200–205°, with 7.2 atoms of deuterium per molecule.

As further indication of the location of part of the deuterium, a sample of 30 mg. of dethiobenzylpenicillin, containing 7.4 atoms of deuterium per molecule, was hydrolyzed by heating under reflux with 10% hydrochloric acid for 3 hours. From the hydrolysate was isolated 8 mg. of phenylacetic acid, micro m.p. 71–75°, containing 4.2 atoms of deuterium per molecule.

¹*Organic Syntheses*, Vol. 21, Wiley, New York, 1941, p. 15.

²R. Mozingo, D. E. Wolf, S. A. Harris and K. Folkers, *J. Am. Chem. Soc.*, **65**, 1013 (1943).

DIMETHYLMERCURY-H₃²



E. E. Bevege, R. Renaud and L. C. Leitch, *Can. J. Chem.*, **31**, 1259 (1953).

A. Procedure (Note 1)

Cadmium amalgam is prepared by shaking 13.0 g. of cadmium turnings and 20 ml. of redistilled mercury in a bottle. The amalgam is poured into a constricted 12 × 1-inch tube, which is then attached to a vacuum line. Methyl-H₃² iodide, 12.5 g., is distilled into the evacuated tube which is then sealed at the constriction. The reaction mixture is heated for 1 day at 100° and 3 days at 125° (Note 2). The volatile material is distilled (vacuum line) into a cold trap and then fractionally distilled at 0°, under vacuum, into other traps on the line. After a 0.1-ml. fraction with vapor pressure of 19.5 mm. at 0° is collected, the main fraction, 2.2 ml., has a constant vapor pressure of 17.9 mm. at 0° (Note 3).

B. Notes

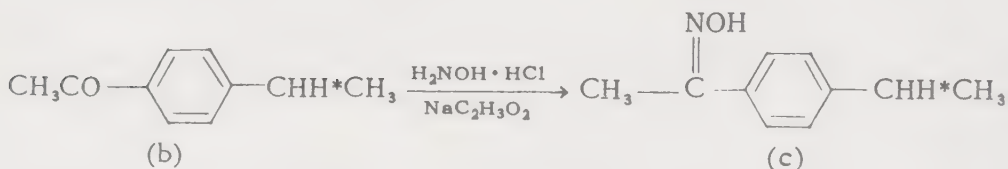
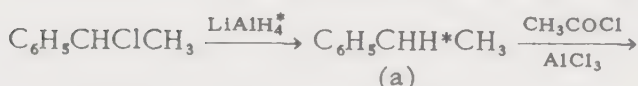
1. Although several methods have been reported¹ for the preparation of dimethylmercury, none of them is suitable when economy of deuterated intermediates is of importance. Dimethylmercury- H_2^2 is readily prepared by an adaptation of the method of Emeléus and Haszeldine² for the synthesis of organofluorine compounds.

2. There was no pressure in the cold tube when it was opened.

3. Mass spectrometric analysis indicated no methyl- H_3^2 iodide in the product.

¹J. Schmidt, *Organometallverbindungen II*, 1934, 160.

²H. J. Emeléus and R. N. Haszeldine, *J. Chem. Soc.*, 1949, 2953.

4'-ETHYL-1- H_1^2 -ACETOPHENONE OXIME

E. L. Eliel, *J. Am. Chem. Soc.*, 71, 3970 (1949).

A. Procedure

(a) *Ethyl-1- H_1^2 -benzene* (Note 1). To a well-stirred mixture of 75 ml. of tetrahydrofuran (Note 2), 1 g. of lithium aluminum hydride- H_2^2 ¹ and 3 g. of finely-ground (100 mesh) lithium hydride- H^2 ¹ is added rapidly 33.5 g. of (-)-(1-chloroethyl)benzene (Note 3). The mixture is refluxed with efficient stirring for 4 hours, without stirring for 9.5 hours and with stirring for 10 hours more. The mixture is cooled, excess lithium aluminum hydride- H_2^2 is destroyed by the addition of a solution of 20 ml. of water in 30 ml. of tetrahydrofuran, and the mixture is poured into ice-water containing 20 ml. of sulfuric acid. The product is extracted with two 100-ml. portions of pentane; the solution is washed twice with water, four times with 85% phosphoric acid, twice with water, once with 10% sodium carbonate solution and again with water. The extract is dried over calcium chloride and fractionated (Note 4). The product is collected at 133-135° (747 mm.); yield, 20.7 g (79%), $\alpha_D^{25} - 0.51 \pm 0.01^\circ$ ($l = 2$ dm., no solvent). This product is refractionated through an 8-inch helix-packed column (Note 5).

(b) *4'-Ethyl-1- H_1^2 -acetophenone*. According to the method of Klages,² 19 g. of aluminum chloride is added gradually with stirring to a solution of 14.5 g. of ethyl-1- H_1^2 -benzene and 18 g. of acetyl chloride in 60 ml. of

petroleum ether. The mixture is refluxed for 0.5 hour and poured onto ice hydrochloric acid. The water layer is extracted with ether and which is combined with the petroleum ether solution and washed successively with dilute hydrochloric acid, water, 10% sodium carbonate solution and water. After the solution is dried over sodium sulfate, and the solvent is removed, fractionation of the residue *in vacuo* yields 1.3 g. of unchanged ethyl-1- H_1^2 -benzene, b.p. 37–38° (17 mm.) and 14.5 g. (72%) of the desired product, b.p. 123–126.5° (18 mm.), n_D^{25} 1.5260, α_D^{23} 0.54 \pm 0.01° (l = 2 dm., no solvent). Assuming a density of 0.99,³ $[\alpha]_D^{23}$ –0.29°.

(c) 4'-Ethyl-1- H_1^2 -acetophenone Oxime. Sodium acetate trihydrate, 29 g., and hydroxylamine hydrochloride, 14.5 g., are ground together in a mortar, and the resultant slurry is extracted 3 times with 50-ml. portions of ethanol. The filtered ethanol solution is added to a solution of 14 g. of 4'-ethyl-1- H_1^2 -acetophenone in 15 ml. of ether and refluxed for 12 hours. Part of the solvent is then distilled, water is added to the cloud point, and crystallization is induced by cooling the solution.

The crystalline oxime is collected, washed with dilute ethanol and dried; yield, 13.2 g. (85%), m.p. 82.5–84°. Recrystallization of the product from dilute ethanol raises the m.p. to 83–89° (Note 6).

B. Notes

1. The general method of Johnson³ for the reduction of alkyl halides to alkanes was followed.

2. The tetrahydrofuran was distilled from potassium hydroxide and then from lithium aluminum hydride. All solvents used in this work were checked for optical rotation, which in no case was found to exceed 0.01° in a 2-dm. tube.

3. The (-)-(1-chloroethyl)benzene used had $[\alpha]_D^{25}$ –49.2°. Eliel gives the preparation of DL- α -methylbenzyl alcohol, resolution of this alcohol into the D- and L- forms and conversion of the latter into D- and L-(1-chloroethyl)benzene.

4. A Vigreux column was used and a small amount of DL-ethyl-1- H_1^2 -benzene was found in the residue.

5. The following fractions were collected:

Fraction	Weight, g.	n_D^{25}	α_D^{25} , degrees
1	5.5	1.4921	–0.47 \pm 0.02
2	3.9	1.4925	–0.48 \pm 0.02
3	4.4	1.4923	–0.53 \pm 0.02
4	4.6	1.4922	–0.52 \pm 0.02
5	0.9	1.4922	Not observed

Fractions 1-4 were recombined and their rotation was found to be $\alpha_D^{25} -0.52 \pm 0.01^\circ$ ($l = 2$ dm., no solvent); taking $d = 0.87$ (0.8712 was observed and the formula of McLean and Adams⁴ gives 0.9810), $[\alpha]_D^{25} -0.30^\circ$. The compound gave negative halogen tests.

6. Further recrystallization from the same solvent did not change the melting point; $[\alpha]_D^{28} -0.12 \pm 0.01^\circ$ ($l = 2$ dm., in benzene); after recrystallization from benzene-petroleum ether, $\alpha_D^{29} -0.11 \pm 0.01^\circ$ ($l = 2$ dm., in benzene), whence $[\alpha]_D^{28} -0.17^\circ$ in benzene. Hydrolysis of the oxime with 1 *N* hydrochloric acid gave 85% recovery of the ketone b.p. 127-129° (25 mm.); $n_D^{25} 1.5261$, $d_4^{25} 0.9915$, $\alpha_D^{30} -0.50 \pm 0.02^\circ$ ($l = 2$ dm., no solvent).

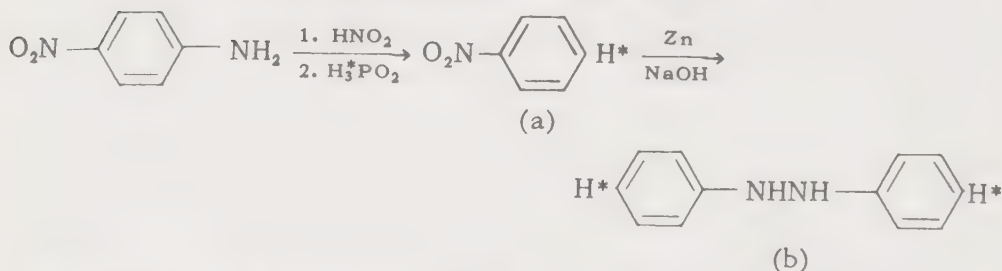
¹Metal Hydrides, Inc., Beverly, Mass.

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HYDRAZOBENZENE-4,4'-H₂²



G. S. Hammond and W. Grundemeir, J. Am. Chem. Soc., 77, 2444 (1955).

A. Procedure

(a) *1-Nitrobenzene-4-H²*. 4-Nitroaniline, 13.8 g., is dissolved in a mixture of 40 ml. of concentrated hydrochloric acid in 50 g. of water-H₂². This solution is cooled to -5° , and a solution of 7.2 g. of sodium nitrite in 17.5 ml. of water is added during 1 hour. The solution is filtered and again cooled to -5° . Precooled hypophosphorous acid-H₂² (Note 1) is added to the diazonium salt solution at a rate such that the temperature of the solution is maintained below 0° . The resulting solution is stored in a refrigerator for 40 hours. Then the solution is extracted with ether, and the extract is washed with aqueous sodium hydroxide. After the ether solution is dried, it is concentrated on a water-bath. Distillation of the residue gives 5.04 g. (42%) of 1-nitrobenzene-4-H².

(b) *Hydrazobenzene-4,4'-H₂²*. 1-Nitrobenzene-4-H², 4.4 g., is reduced with zinc dust and alkali according to the method of Fischer¹ (Note 2). After recrystallization from hot ethanol, the yield of hydrazobenzene-4,4'-H₂², m.p. 128-129°, is 2.84 g. (86%).

B. Notes

1. Hypophosphorous acid- H_2^2 was prepared by modification of the procedure of Alexander and Burge² (see 1-nitrobenzene-3- H^2). Commercial 30% hypophosphorous acid is concentrated, below 100° under vacuum, to 99%. The concentrated acid, 44 ml., is added to 50 g. of water- H_2^2 , and this solution is kept at room temperature for 30 hours. The solution is concentrated again, and the residue is treated with another portion of water- H_2^2 . After the final concentration of the hypophosphorous acid- H_2^2 , infrared analysis indicated that not less than 60% exchange of hydrogen had occurred.

2. A detailed procedure for this reduction is presented by Hickinbottom.³

¹E. Fischer, *Anleitung zur Darstellung organischen Präparate*, Williams and Norgate, London, 1909, p. 41.

²E. R. Alexander and R. E. Burge, Jr., *J. Am. Chem. Soc.*, 72, 3100 (1950).

³W. J. Hickinbottom, *Reactions of Organic Compounds*, Longmans, Green and Co., New York, 1936, p. 320.

TABLE XVI, 12
 Deuterium Exchange with Acids

Formula	Acid	Isotope Source	Catalyst	Temp., °C.	Time	Notes	Ref.
CH_2O_2	Formic	H_2^2O	room	Product contained 3-4% HCOOH .	1
CH_2O_2	Formic	H_2^2O	room	The product was dried with anhydrous copper sulfate.	2
CH_2O_2	Formic	H_2^2O	room	The product was dried by distillation from boric oxide at room temperature under reduced pressure.	3
CH_2O_2	Formic	H_2^2O	room	Exchange of carboxyl hydrogen only.	4
$\text{C}_2\text{H}_2\text{O}_4$	Oxalic	H_2^2O	Repeated recrystallization of anhydrous oxalic acid from water- H_2^2 gave $(\text{COOH}^2)_2 \cdot 2\text{H}_2^2\text{O}$, m.p. 95.9-97.5°.	5,6
$\text{C}_2\text{H}_2\text{O}_4$	Oxalic	H_2^2O	Equilibration of anhydrous oxalic acid with water- H_2^2 .	7
$\text{C}_2\text{H}_2\text{O}_4$	Oxalic	H_2^2O	Solution of acid in H_2^2O evaporated under vacuum.	32
$\text{C}_2\text{H}_3\text{ClO}_2$	Chloroacetic	H_2^2O	Exchange of carboxyl hydrogen only.	4
$\text{C}_2\text{H}_3\text{ClO}_2$	Chloroacetic	H_2^2O	room	Ionization constant in water- $\text{H}_2^2 = 0.63 \times 10^{-3}$.	8
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	CH_3COONa	25-30	14 days	No measurable exchange.	9
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	CH_3COOH^2	150	100 hrs.	H atoms of methyl group, 30% of equilibrium.	35
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	CH_3COONa	66 hrs.	Apparently 34% of methyl hydrogen exchanged; temperature not given.	10
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	alkali	Exchange (15%) and hydrolysis of acetamide.	11,13
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	alkali	Exchange (15%) and hydrolysis of acetamide.	12
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	alkali	125-130	Exchange, 2.9 atom % deuterium per day.	14
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	Pt	125-130	Exchange, 5.4 atom % deuterium per day.	14
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2	Pt	30	Exchange was completely inhibited by addition of 1% nitrobenzene; as much as 50% nitroethane had no effect.	15
$\text{C}_2\text{H}_4\text{O}_2$	Acetic	H_2^2O	NaOH	100	400 hrs.	Exchange number, 0.407.	16

$C_2H_4O_2$ $C_2H_4O_2$	Acetic Acetic	H_2O H_2	NaOH Pt	100 $35 \pm 0.2^\circ$	400 hrs. 3 min.	Mixture of pure acetic acid and water- H_2 . Compared to the rate of exchange with pure acetic acid, addition of benzene, aniline and nitromesitylene reduced the rate; with the latter two solutes a limiting concentration was found. β -Nitrostyrene and 2-nitro-1-butene caused an increase in rate at low concentrations but increase in concentration finally reduced the rate to zero. Oximes caused increase in rate which reached a maximum with concentration. The inhibitory effect of nitro compounds disappeared as the nitro groups were reduced.	17 30
$C_2H_4O_3$ $C_3H_4O_2$	Glycolic Acrylic	H_2O H_2O 5 moles % excess NaOH	190-210 100 100 hrs.	Half time of exchange, 330 hours. Exchange number, 0.365	18 16
$C_3H_4O_4$ $C_3H_6O_2$	Malonic Propionic	H_2O H_2O 5 moles % excess NaOH 100 400 hrs.	Solution of acid in H_2O evaporated under vacuum. Exchange number, 0.039	32 16
$C_3H_6O_2$	Propionic	H_2O	Pt	125-130	2-10 days	With concentrations of acid and water 10 molar, 1.1 atom % of deuterium was introduced per day. With catalyst present, deuterium entered both α - and β -positions; without catalyst, only the α -position.	14
$C_3H_6O_3$	Lactic	H_2O	Pt	125-130	2-10 days	Exchange rate was proportional to amount of catalyst.	14
$C_4H_4O_4$	Acetylene-dicarboxylic	H_2O	Acetylenedicarboxylic acid- $H_2 \cdot H_2O$ was obtained by repeated recrystallization of the anhydrous acid.	31

(Continued)

TABLE XVI, 12 (Continued)

Formula	Acid	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₄ H ₃ O ₄	Acetylene-dicarboxylic	H ₂ O	Solution of acid in H ₂ O evaporated under vacuum.	32
C ₄ H ₄ O ₄	Maleic	H ₂ O	H ₂ O was distilled from solution of maleic acid.	33
C ₄ H ₆ O ₂	3-Butenoic	H ₂ O	N NaOH	100	2 hrs.	Exchange of one of carbon-bound hydrogen atoms.	19
C ₄ H ₆ O ₂	3-Butenoic	H ₂ O	N NaOH	100	3 hrs.	Exchange number (apparent number of hydrogen atoms exchanged) = 0.934. Exchange is faster than isomerization to crotonic acid.	20
C ₄ H ₆ O ₂	Crotonic	H ₂ O	N NaOH	100	2 hrs.	No exchange.	19
C ₄ H ₆ O ₄	Succinic	H ₂ O	20 g. NaOH per 100 ml.	reflux	48 hrs.	Succinic acid had 27.59 atom % deuterium.	21
C ₄ H ₆ O ₄	Succinic	H ₂ O	Partition coefficient, 0.98 ± 0.09.	22
C ₄ H ₆ O ₄	Succinic	H ₂ O	70	2-4 hrs.	Exchange of carboxyl hydrogen atoms; coefficient = 1.	23
C ₄ H ₆ O ₄	Succinic	H ₂ O	Solution of acid in H ₂ O evaporated under vacuum.	32
C ₄ H ₈ O ₂	Butyric	H ₂ O	100	405 hrs.	No exchange in acidic medium; exchange doubtful in basic medium.	16
C ₄ H ₈ O ₂	Isobutyric	H ₂ O	NaOH	100	400 hrs.	Exchange doubtful.	16
C ₄ H ₈ O ₃	2-Methoxypropionic	H ₂ O	Pt	125-130	Exchange slight, 0.04 atom % deuterium per day.	14
C ₃ H ₈ O ₂	2-Pentenoic	H ₂ O	Sodium salt of acid	100	Rapid exchange of the α-hydrogen atoms was assumed. A 3-component equilibrium system was established between 2-pentenoic acid, 3-pentenoic acid and 3-hydroxypentanoic acid.	24
C ₃ H ₈ O ₂	3-Pentenoic	H ₂ O	Sodium salt of acid	100	See 2-pentanoic.	24
C ₅ H ₈ O ₄	Glutaric	H ₂ O	Solution of acid in H ₂ O evaporated under vacuum.	32
C ₅ H ₁₀ O ₂	Pivalic	H ₂ O	Solution of acid in H ₂ O evaporated under vacuum.	32

$C_3H_{10}O_2$	Pivalic	H_2O	Pt	125-130	...	Exchange slight, 0.18 atom % deuterium per day.	14
$C_3H_{10}O_3$	3-Hydroxy-pentanoic	H_2O	Sodium salt of acid	100	...	See 2-pentanoic.	24
$C_6H_8O_2$	Sorbic	H_2O	5 moles % excess NaOH	100	100 hrs.	Exchange number, 0.493.	16
$C_6H_{10}O_4$	Adipic	H_2O	20 moles KOH per mole acid	reflux	24 hrs.	Atom % deuterium, 0.11; other than carboxyl.	25
$C_6H_{10}O_4$	Adipic	H_2O	Solution of acid in H_2O evaporated under vacuum.	32
$C_7H_6O_2$	Benzoic	H_2O	5 moles % excess NaOH	100	100 hrs.	Exchange number, 0.004.	16
$C_7H_6O_2$	Benzoic	H_2O	Partition coefficient 1.04.	34
$C_7H_7NO_2$	Anthranilic	H_2O	Distribution coefficient, 1.01.	22
$C_7H_{12}O_2$	Cyclohexanecarboxylic	H_2O	Solution of acid in H_2O evaporated under vacuum.	32
$C_8H_8O_2$	Phenylacetic	H_2O	5 moles % excess NaOH	100	100 hrs.	Exchange number, 1.765.	16
$C_8H_8O_2$	<i>p</i> -Toluic	H_2O	5 moles % excess NaOH	100	100 hrs.	Exchange number, 0.006.	16
$C_8H_8O_3$	Mandelic	H_2O	...	60	...	No racemization, 2 hydrogen atoms exchanged in recrystallization from water- H_2^2 .	26,27
$C_8H_8O_3$	Mandelic	H_2O	...	140	51 hrs.	Complete racemization; 2 hydrogen atoms exchanged.	26,27
$C_8H_8O_3$	Mandelic	H_2O	$NaOH^2$	100	16 hrs.	Complete racemization; 2.34 hydrogen atoms exchanged.	26,27
$C_8H_8O_3$	L-Mandelic	H_2O	...	60	...	No racemization but a significant change in optical activity.	28

(Continued)

TABLE XVI, 12 (Continued)

Formula	Acid	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_8H_{14}O_4$	Suberic	$H_2^{18}O$	20 moles KOH per mole acid	reflux	24 hrs.	Atom % deuterium, 0.06; other than carboxyl.	25
$C_9H_8O_2$	Cinnamic	$H_2^{18}O$	5 mole % excess NaOH	100	100 hrs.	Exchange number, 0.015.	16
$C_9H_{10}O_2$	Hydratropic	$H_2^{18}O$	5 mole % excess NaOH	100	100 hrs.	Exchange number, 0.063.	16
$C_9H_{10}O_2$	Hydrocinnamic	$H_2^{18}O$	5 mole % excess NaOH	100	100 hrs.	Exchange number, 0.032.	16
$C_9H_{10}O_3$	D-Atrolactic	$H_2^{18}O$	No racemization but a significant change in rotation.	28
$C_{10}H_{18}O_4$	Sebacic	$H_2^{18}O$	20 molar KOH or 1 N H_2SO_4	reflux	24 hrs.	Atom % deuterium in product, 0.03.	25
$C_{12}H_{24}O_2$	Lauric	$H_2^{18}O$	Solution of acid in $H_2^{18}O$ evaporated under vacuum.	32
$C_{15}H_{14}O_2$	Phenyl- <i>p</i> -tolylacetic	$H_2^{18}O$	NaOH ²	100	2 days	Two such treatments gave a product which was resolved into D- and L- forms having respectively, 99.9 and 98.5% deuterium in the α -position.	29
$C_{18}H_{36}O_2$	Stearic	$H_2^{18}O$	Solution of acid in $H_2^{18}O$ evaporated under vacuum.	32

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TABLE XVI, 13
Deuterium Exchange with Alcohols

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
CH ₄ O	Methanol	H ₂ O	A continuous distillation process for 1 compounds more volatile than water was described.	1
CH ₄ O	Methanol	1 mole	H ₂ S	9 moles	-79	~0.2 min.	Half time of exchange to equilibrium was about 0.2 min. Product was 66 atom % methanol-H ² .	2
CH ₄ O	Methanol	1 mole	H ₂ O	1 mole	80	Equilibrium constant = 0.481 ± 0.015.	3
CH ₄ O	Methanol	1 mole	butanol-H ²	1 mole	b.p.	2 hrs.	Exchange occurs.	4
CH ₄ O	Methanol	H ₂ O	Pt black	No exchange in methyl group. Equal distribution of deuterium between water and methanol.	5
CH ₄ O	Methanol	3.22 mm.	H ₂	12.9 mm.	Ni film	74	Deuterium distribution: methanol-H ² (76%), methanol-H ² -1-H ₂ ² (14%), methanol-H ² -1-H ₂ ² (9.5%) and methanol-H ₂ ² (0.5%).	18
CH ₄ O	Methanol	3.22 mm.	H ₂	12.9 mm.	Fe film	112	Deuterium distribution: methanol-H ² (93.9%), methanol-H ² -1-H ₂ ² (5.6%), methanol-H ² -1-H ₂ ² (0.5%) and methanol-H ₂ ² (0.0%); k = 1.04%/min.	18
CH ₄ O	Methanol	3.22 mm.	H ₂	12.9 mm.	W film	88	Deuterium distribution: methanol-H ² (86.1%), methanol-H ² -1-H ₂ ² (7.7%), methanol-H ² -1-H ₂ ² (4.6%) and methanol-H ₂ ² (1.6%); k = 0.38%/min.	18
CH ₄ O	Methanol	3.22 mm.	H ₂	12.9 mm.	Rh film	38.5	Deuterium distribution: methanol-H ² (84.4%), methanol-H ² -1-H ₂ ² (8.9%), methanol-H ² -1-H ₂ ² (5.9%) and methanol-H ₂ ² (1.8%); k = 3.6%/min.	18

CH ₄ O	Methanol	3.22 mm.	H ₂ ²	12.9 mm.	Pd film	51	Deuterium distribution: methanol-H ² (98.6%), methanol-H ² -1-H ₁ ² (1.4%).	18
CH ₄ O	Methanol	3.22 mm.	H ₂ ²	12.9 mm.	Ag film	262	Deuterium distribution: methanol-H ² (76.0%), methanol-H ² -1-H ₁ ² (14.1%), methanol-H ² -1-H ₂ ² (7.1%) and methanol-H ₄ ² (2.8%); k = 1.04%/min.	18
CH ₄ O	Methanol	3.22 mm.	H ₂ ²	12.9 mm.	ZnO	180	Deuterium distribution: methanol-H ² (100%); k = 1.1%/min.	18
CH ₄ O	Methanol	0.0481 mole	H ₂ O	0.0275 mole	Equilibrium constant = 1.82.	21
C ₂ H ₆ O	Ethanol	0.0815	H ₂ O	0.35 mole	25	26 hrs.	Equilibrium constant = 1.11.	6
C ₂ H ₆ O	Ethanol	Ca(OH ²) ₂	room	1 hr.	Equilibrium in less than 1 hour between calcium hydroxide-H ₂ ² and alcohol vapor.	7
C ₂ H ₆ O	Ethanol	115 ml.	H ₂ O	10 ml.	room	37.4 atom % H ² in hydroxyl.	8
C ₂ H ₆ O	Ethanol	4.21 mole	H ₂ O	0.21 mole	room	several hours	Product was dried with calcium oxide and distilled.	9
C ₂ H ₆ O	Ethanol	H ₂ O	10
C ₂ H ₆ O	Ethanol	H ₂ O	11
C ₂ H ₆ O	Ethanol	3.2 ml.	H ₂ O	1 ml.	0	5 sec.	Exchange was very rapid, nearly reaching equilibrium.	12
C ₂ H ₆ O	Ethanol	3.22 mm.	H ₂ ²	19.3 mm.	Rh film	100	Deuterium distribution: ethanol-H ² (95.7%), ethanol-H ² -1-H ₁ ² (0.5%), ethanol-H ² -1-H ₂ ² (1.1%), ethanol-H ² -1,1,2-H ₂ ² (1.0%), ethanol-H ² -1,1,2,2-H ₂ ² (0.9%), ethanol-H ₄ ² (0.8%); k = 2.7%/min.	18
C ₂ H ₆ O	Ethanol	3.22 mm.	H ₂ ²	19.3 mm.	Fe film	151	Deuterium distribution: ethanol-H ² (63%), ethanol-H ² -1-H ₁ ² (32%), ethanol-H ² -1-H ₂ ² (5%), ethanol-H ² -1,1,2-H ₂ ² (0.3%); k = 4%/min.	18

(Continued)

TABLE XVI, 13 (Continued)

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C_2H_6O	Ethanol	3.22 mm.	H_2^1	19.3 mm.	Pt film	40	...	Deuterium distribution: ethanol- H^2 (87%), ethanol- H^2 -1- H_2^1 (9%), ethanol- H^2 -1- H_2^1 (3%), ethanol- H^2 -1,1,2- H_2^1 (1%). Exchange rapid.	18
$C_2H_6O_2$	Ethylene glycol	...	H_2^1O	2%		13
$C_2H_6O_2$	Ethylene glycol	5.8 g.	H_2^1O	2 ml.		14
C_2H_6S	Ethanethiol	0.48 mole	H_2^1O	1.98 mole	...	25	18 hrs.	Equilibrium constant = 0.215.	15
C_3H_8O	2-Propanol	3.35 mm.	H_2^1	13.4 mm.	Pd film	41	...	Product was propanol- H^2 ; k = 2.6%/min.	18
$C_3H_8O_3$	Glycerol	6.065 g.	H_2^1O	2 ml.	Equilibrium in 23 minutes.	14
$C_4H_{10}O$	Butanol	...	Methyl-amine-1- H_3^2 · H^2Cl	117.7	...	Butanol was recrystallization solvent.	16
$C_4H_{10}O$	2-Methyl-2-propanol	3.35 mm.	H_2^1	13.4 mm.	Pd film	79	...	Product was 2-methyl-2-propanol- H^2 ; k = 2.2%/min.	18
$C_4H_{10}O$	2-Methyl-2-propanol	...	H_2^1O	Alcohol was equilibrated, dried and distilled.	19
$C_5H_{12}O$	2-Methyl-2-butanol	...	ethylamine- $N-H_3^2$	Exchange rapid.	19
C_7H_8O	Benzyl alcohol	0.096 mole	H_2^1O	0.11 mole	Partition coefficient, 1.10-	20
$C_8H_{18}O$	Octanol	...	H_2^1O	room	17
$C_{11}H_{24}O$	2,2,6,6-Tetra-methyl-4-heptanol	...	2-methyl-2-propanol- H^2	Exchange rapid.	19

References to Table XVI, 13, DEUTERIUM EXCHANGE WITH ALCOHOLS

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TABLE XVI, 14
Deuterium Exchange with Aldehydes and Ketones

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
CH ₂ O	Formaldehyde	...	H ₂ O	Complete exchange.	1
CH ₂ O	Formaldehyde	...	H ₂ O	...	NaOH	No exchange.	2
C ₂ H ₄ O	Acetaldehyde	...	H ₂ O	Exchange of 1 H atom, slowly.	1
C ₃ H ₆ O	Acetone	...	H ₂ O	...	0.6 M NaOH	room	18 hrs.	Complete exchange of all H atoms to equilibrium.	4
C ₃ H ₆ O	Acetone	...	H ₂ O	5
C ₃ H ₆ O	Acetone	...	H ₂ O	...	NaOH	Rapid, complete exchange.	1
C ₃ H ₆ O	Acetone	60 ml.	H ₂ O	30 ml.	0.1 g. K ₂ CO ₃	steam-bath	1-3 hrs.	41-52% exchange.	6
C ₃ H ₆ O	Acetone	...	H ₂ O	7
C ₃ H ₆ O	Acetone	...	H ₂ O	...	base	Rapid exchange.	8
C ₃ H ₆ O	Acetone	...	H ₂ O	...	H ₂ SO ₄	94% exchange in 3 treatments.	9
C ₃ H ₆ O	Acetone	...	H ₂ O	room	...	$\alpha = \left(\frac{H^2}{H}\right)_{\text{water}} \div \left(\frac{H^1}{H}\right)_{\text{solute}} = 1.21$	10
C ₃ H ₆ O	Acetone	...	H ₂ O	...	NaOH	Exchange velocity is proportional to the OH ⁻ concentration.	11
C ₃ H ₆ O	Acetone	...	H ₂ O	Coefficient of exchange = 1.21.	12
C ₅ H ₈ O	Cyclopentanone	...	H ₂ SO ₄	room	1 hr.	Exchange of α -H atoms, 102.9%.	18
C ₅ H ₈ O	Cyclopentanone	...	H ₃ PO ₄	room	46 hrs.	Exchange of α -H atoms, 104.9%.	18
C ₅ H ₈ O ₂	2,4-Pentanedione	...	H ₂ O	17	70 hrs.	Dioxane solvent; approx. 2 H atoms exchanged.	13
C ₅ H ₈ O ₂	2,4-Pentanedione	...	H ₂ S	Reaction of H ₂ S with the copper salt in benzene.	16
C ₅ H ₈ O ₂	2,4-Pentanedione	...	H ₂ O	...	NaOH	Complete exchange, rapid in basic medium.	1

$C_6H_8O_5$	Ethyl oxalacetate	...	H_2^2S	...	NaOH	Reaction of H_2^2S with the copper salt in benzene.	16
$C_6H_{10}O$	Cyclohexanone	...	$H_2^2SO_4$	room	1 hr.	Exchange of α -H atoms, 82.6%.	18
$C_6H_{10}O$	Cyclohexanone	...	$H_2^2PO_4$	room	46 hrs.	Exchange of α -H atoms, 100.0%.	18
$C_6H_{10}O$	Cyclohexanone	...	H_2^2O	100	50 hrs.	Exchange of 4 H atoms.	13
$C_6H_{12}O$	2-Hexanone	...	$H_2^2SO_4$	1 hr.	Exchange of α -H atoms, 99.5%.	18
$C_6H_{12}O$	2-Hexanone	...	$H_2^2PO_4$	room	46 hrs.	Exchange of α -H atoms, 100.3%.	18
C_7H_6O	Benzaldehyde	...	H_2^2O	0.11 mole	...	60	100 hrs.	No exchange.	3
C_8H_8O	Acetophenone	0.0426 mole	$C_2H_5OH^2$	0.0858 mole	2 mg. NaOH	110	96 hrs.	Exchange number = 2.15.	14
$C_9H_{16}O$	2,2,6-Trimethylcyclohexanone	...	H_2^2O	130	70 hrs.	Complete exchange of 1 H atom.	13
$C_{10}H_8O_4$	Oxalacetophenone	...	H_2^2S	Reaction of H_2^2S with the copper salt in benzene.	16
$C_{10}H_{10}O_2$	1-Phenyl-1,3-butanedione	...	H_2^2S	Reaction of H_2^2S with the copper salt in benzene.	16
$C_{10}H_{13}O$	Eucarvone	...	$C_2H_5OH^2$...	C_2H_5ONa	room	...	3 H atoms exchanged.	17
$C_{11}H_{14}O$	L-2-Methylbutyrophenone	1.463 g.	H_2^2O (99.16%)	9.0 g.	NaOH ²	35	6.5 hrs.	Each sample contained 20.0 ml. of dry dioxane. Exchange 57.1% complete.	15
$C_{17}H_{12}O_3$	1,5-Diphenyl-1,3,5-pentanetrione	...	H_2^2S	Reaction of H_2^2S with the copper salt in benzene.	16

References to Table XVI, 14, DEUTERIUM EXCHANGE WITH ALDEHYDES AND KETONES

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TABLE XVI, 15, Deuterium Exchange with Aliphatic Halides

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
CHBr ₃	Bromoform	0.483 mole	CCl ₃ H ²	0.322 mole	butyl-amine	30	13 days	Exchange, 46%.	1
CHBr ₂ Cl	Dibromochloro-methane	0.1 mole	H ₂ O	0.1 mole	0.01 mole NaOH ²	105	4 days	Two treatments of the same sample gave 64% of CH ² Br ₂ Cl.	2
CHBrCl ₂	Bromodichloro-methane	0.1 mole	H ₂ O	0.1 mole	0.01 mole NaOH ²	105	4 days	Two treatments of the same sample gave 36% of CH ² BrCl ₂ .	2
CHCl ₃	Chloroform	H ₂ O	alkali	Exchange in alkaline solution much faster than decomposition.	3
CHCl ₃	Chloroform	12 g.	H ₂ O	2.0 g.	1.38 g. K ₂ CO ₃	100-105	13 days	4
C ₂ HCl ₃	Trichloro-ethylene	1.12 moles	H ₂ O	1.36 moles	6 N NaOH ²	81-84	Four successive exchanges gave 91% C ₂ H ² Cl ₃ .	8
C ₂ H ₅ Br	Bromoethane	0.0081 mole/l.	H ² Br	0.005 mole/l.	306	6 hrs.	Exchange, 94% of equilibrium.	5
C ₃ HF ₃	3,3,3-Tri-fluoropropene	H ₂ O	NaOH ²	room	~ 12 hrs.	About 50% exchange.	6
C ₆ H ₆ Cl ₆	β-Hexachloro-cyclohexane	0.00036	C ₂ H ₅ OH ²	C ₂ H ₅ ONa	43.6	28.5 hrs.	After purification, the β-hexachlorocyclohexane contained 0.077-0.081 atom % H ² .	7

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TABLE XVI, 16
 Deuterium Exchange with Alkanes

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
CH ₄	Methane	14.4 mm.	H ₂ ¹	3.19 mm.	16.9 mg. Ni	224.8	65 min.	8% of total methane was CH ₄ ² .	1
CH ₄	Methane	...	H ₂ ¹	...	Cobalt-thoria-magnesia-kieselguhr	183	17 hrs.	11% exchange, only 1.7% CH ₄ ² .	2
CH ₄	Methane	0.5 mm.	H ² atoms	360	...	H ² atoms from Wood's discharge tube reacted with methane; product was 50% CH ₄ ² , 8% CHH ₃ ² , 3% CH ₃ H ² and 39% CH ₄ . ⁴	3
CH ₄	Methane	6.46 mm.	H ₂ ¹	4.85 mm.	11.1 mg. Ni	237	45 min.	9% of total methane was CHH ₃ ¹ .	1
CH ₄	Methane	6.46 mm.	H ₂ ¹	4.85 mm.	11.1 mg. Ni	237	45 min.	7% of total methane was CH ₃ H ₂ ¹ .	1
CH ₄	Methane	...	H ² atoms	H ² atoms from Wood's discharge tube reacted with acetone; 55% methane in product, 97% deuterized.	4
CH ₄	Methane	40-300	...	Illuminated by mercury resonance radiation. Product was mixture of deuterated methanes.	5
CH ₄	Methane	100 mm.	H ₂ ¹	100 mm.	...	560	1000 min.	Product contained 11.2 mm. of methane-H ₂ ¹ . Methane-H ₂ ¹ was also formed from butane and 1-butene under like conditions.	42
CH ₄	Methane	...	H ₂ ¹	...	Ni	218	50 hrs.	Equilibrium was established.	6
CH ₄	Methane	propane, 0.35 mm.	H ² atoms	30	...	H ² atoms from Wood's discharge tube. Product methane and propane. Methane 56.5% deuterized.	7

CH ₄	Methane	0.5 mm.	H ² atoms	6.0 mm.	310	Wood's discharge tube; 9.6% exchange.	8
CH ₄	Methane	44.0 ml.	H ₂ ²	43.2 ml.	392	2 hrs.	Illuminated by mercury vapor lamp; 15% H ² -methanes.	9
CH ₄	Methane	CH ₄ ²	0.450 g. silica-alumina	345	Mixture of H ² -methanes resulted.	10
CH ₄	Methane	10 cm.	H ₂ ²	45 cm.	23	119 hrs.	Illuminated by Hanovia mercury resonance lamp. Methane had 11.5% H ² content.	11
CH ₄	Methane	6.24 mm.	H ₂ ²	4.65 mm.	Ni	341	90 min.	32% of total methane was CH ₃ H ² .	1
CH ₄	Methane	6.45 mm.	H ₂ ²	6.45 mm.	1.8 mg. Rh film	162.0	20 min.	Product: methane-H ₁ ² , 0.7%; -H ₂ ² , 0.1%; -H ₃ ² , 0.9%; -H ₄ ² , 2.1%.	38
CH ₄	Methane	6.45 mm.	H ₂ ²	6.45 mm.	2.3 mg. Pt film	259.3	20 min.	Product: methane-H ₁ ² , 13.5%; -H ₂ ² , 5.9%; -H ₃ ² , 5.5%; -H ₄ ² , 4.5%.	38
CH ₄	Methane	6.45 mm.	H ₂ ²	6.45 mm.	8.5 mg. W film	150.6	15 min.	Product: methane-H ₁ ² , 15.9%; -H ₂ ² , 2.2%; -H ₃ ² , 1.4%; -H ₄ ² , 1.5%.	38
CH ₄	Methane	6.45 mm.	H ₂ ²	6.45 mm.	3.5 mg. Pd film	254.3	20 min.	Product: methane-H ₁ ² , 13.0%; -H ₂ ² , 5.5%; -H ₃ ² , 0.25%; -H ₄ ² , 0.25%.	38
C ₂ H ₆	Ethane	H ₂ ²	Ni wire	90	Deuterium exchange with ethylene was followed by H ₂ ² addition.	12
C ₂ H ₆	Ethane	H ² atoms	170	H ² atoms from Wood's discharge tube reacted with propane; highly deuterized ethane formed above 100°.	7
C ₂ H ₆	Ethane	H ² atoms	208	H ² atoms from Wood's discharge tube; ethane was 15.7% decomposed; 136% exchange of H.	8
C ₂ H ₆	Ethane	H ² atoms	20	H ² atoms from Wood's discharge tube; H ² content of ethane, 19.8%.	11

(Continued)

TABLE XVI, 16 (Continued)

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C_2H_6	Ethane	Ni	138	Exchange of H^2 with ethane quantitative; product $C_2H_4H^2$.	13
C_2H_6	Ethane	2.90 mm.	H^2_2	11.60 mm. W film		-80 to -29	H^2 distribution: H^2_1 , 78%; H^2_2 , 12%; H^2_3 , 5.1%; H^2_4 , 2.0%; H^2_5 , 0.9%; H^2_6 , 0.6%.	39
C_2H_6	Ethane	2.32 mm.	H^2_2	17.56 mm. Mo film		-50 to 0	H^2 distribution: H^2_1 , 87%; H^2_2 , 14%; H^2_3 , 3.0%; H^2_4 , 0.7%; H^2_5 , 0%; H^2_6 , 0%.	39
C_2H_6	Ethane	2.32 mm.	H^2_2	17.56 mm. Ta film		-44 to 0	H^2 distribution: H^2_1 , 81%; H^2_2 , 15%; H^2_3 , 3.1%; H^2_4 , 0.6%; H^2_5 , 0%; H^2_6 , 0%.	39
C_2H_6	Ethane	2.32 mm.	H^2_2	17.56 mm. Zr film		158 to 192	H^2 distribution: H^2_1 , 52%; H^2_2 , 17%; H^2_3 , 5.1%; H^2_4 , 4.3%; H^2_5 , 7.0%; H^2_6 , 14%.	39
C_2H_6	Ethane	2.58 mm.	H^2_2	20.64 mm. Cr film		149 to 215	H^2 distribution: H^2_1 , 27%; H^2_2 , 18%; H^2_3 , 6.6%; H^2_4 , 6.0%; H^2_5 , 7.2%; H^2_6 , 15%.	39
C_2H_6	Ethane	2.58 mm.	H^2_2	20.64 mm. V film		102 to 160	H^2 distribution: H^2_1 , 46%; H^2_2 , 19%; H^2_3 , 5.7%; H^2_4 , 5.1%; H^2_5 , 7.5%; H^2_6 , 16%.	39
C_2H_6	Ethane	2.58 mm.	H^2_2	20.64 mm. Ni film		162 to 195	H^2 distribution: H^2_1 , 40%; H^2_2 , 10%; H^2_3 , 4%; H^2_4 , 5%; H^2_5 , 10%; H^2_6 , 30%.	39
C_2H_6	Ethane	2.58 mm.	H^2_2	20.64 mm. Pt film		134 to 192	H^2 distribution: H^2_1 , 19%; H^2_2 , 17%; H^2_3 , 12%; H^2_4 , 10%; H^2_5 , 15%; H^2_6 , 25%.	39
C_2H_6	Ethane	2.58 mm.	H^2_2	20.64 mm. Pd film		145 to 207	H^2 distribution: H^2_1 , 5%; H^2_2 , 6%; H^2_3 , 8%; H^2_4 , 11%; H^2_5 , 19%; H^2_6 , 52%.	39

C_2H_6	Ethane	2.58 mm.	H_2^2	20.64 mm. Rh film	0 to 70	H_2^2 distribution: H_1^2 , 0%; H_2^2 , 0%; H_3^2 , 5%; H_4^2 , 10%; H_5^2 , 20%; H_6^2 , 60%.	39
C_2H_6	Ethane	2.58 mm.	H_2^2	20.64 mm. Co film	Cobalt more active for cracking than for exchange.	39
C_2H_6	Ethane	2.58 mm.	H_2^2	20.64 mm. Fe film	Iron more active for cracking than for exchange.	39
C_3H_8	Cyclopropane	5.0 mm.	H_2^2	224.0 mm. 5% Pt-pumice-0.5 g.	200	H_2^2 distribution: C_3H_6 , 7.0%; $C_3H_7H^2$, 1.8%; $C_3H_6H_2^2$, 6.7%; $C_3H_5H_3^2$, 8.0%; $C_3H_4H_4^2$, 13.1%; $C_3H_3H_5^2$, 7.1%; $C_3H_2H_6^2$, 5.5%; $C_3H_1H_7^2$, 25.4%; C_3H_8 , 32.5%. Increase in temperature increased deuterium-hydrogen exchange; pressure had little effect.	41
C_3H_8	Propane	24.0 mm.	H_2^2	76.0 mm. 5% Pt-pumice, 5 g.	200	H_2^2 distribution: $C_3H_7H^2$, 11.7%; $C_3H_6H_2^2$, 12.3%; $C_3H_5H_3^2$, 14.3%; $C_3H_4H_4^2$, 17.9%; $C_3H_3H_5^2$, 19.5%; $C_3H_2H_6^2$, 15.3%; $C_3H_1H_7^2$, 7.1%; C_3H_8 , 1.6%.	41
C_3H_8	Propane	H_2^2	Ni	21 hrs.	Seven 3-hour periods of exchange gave 98.9% C—H ² bonds.	14
C_3H_8	Propane	H_2^2	Pt	73 min.	Exchange and hydrogenation of acetone.	15
C_3H_8	Propane	H_2^2	Pt	50 min.	Exchange and hydrogenation of acetone.	16
C_3H_8	Propane	H_2^2	Pt	Exchange practically to equilibrium; product was a mixture of H^2 -propanes.	17
C_3H_8	Propane	propane-1- H_1^2 or propane-2- H_1^2	$AlCl_3$ -on-alumina	25	Intermolecular exchange.	18

(Continued)

TABLE XVI, 16 (Continued)

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₃ H ₈	Propane	H ² atoms	109	H ² atoms from Wood's discharge tube; 50.8% exchange of H ² for H. Propane was 10.6% decomposed.	8
C ₄ H ₁₀	Butane	butane-1 1-H ² ₁ or butane-2 2-H ² ₁	AlCl ₃ -on-alumina	25	5 hrs.	Random distribution of deuterium by intermolecular exchange.	18
C ₄ H ₁₀	Butane	H ² ₁	Pt	66	70 min.	All hydrogen atoms of butane molecule participate equally in the exchange. Product was mixture of H ² -butanes.	17
C ₄ H ₁₀	Butane and 2-Methylpropane	H ² Br	AlBr ₃ -H ² Br	25	20 hrs.	Upon addition of a trace of butene, over 40% of butane isomerized to 2-methylpropane, and 92% of total deuterium exchanged.	21
C ₄ H ₁₀	Butane	H ₂ O	Pd	120	160 hrs.	No exchange.	20
C ₄ H ₁₀	Butane	H ² Br	AlBr ₃ -H ² Br	25	20 hrs.	6% of total deuterium exchanged.	21
C ₄ H ₁₀	Butane	H ² atoms	110	H ² atoms from Wood's discharge tube; 50% exchange of H ² for H; butane was 11.0% decomposed.	8
C ₄ H ₁₀	Butane-1-H ² ₁	H ₂ SO ₄	25	3 hrs.	No exchange of deuterium with acid.	22
C ₄ H ₁₀	Butane-2-H ² ₁	H ₂ SO ₄	25	3 hrs.	No exchange of deuterium with acid.	22

C_4H_{10}	Butane	H_2O	silica- alumina	280	1 hr.	1.91 mole % of product deuterated to some degree.	19
C_4H_{10}	2-Methyl- propane	2-methyl- propane- $2-H^2$	$AlCl_3$ -on- alumina	25	24 hrs.	Random distribution of deuterium by intermolecular exchange. Much less exchange with 2-methylpropane- $1-H^2$.	18
C_4H_{10}	2-Methyl- propane	H^2Br	$AlBr_3$ - H^2Br	25	20 hrs.	9.54% of total deuterium exchanged.	21
C_4H_{10}	2-Methyl- propane	H_2O	silica- alumina	120	1 hr.	6.23 mole % of product deuterated to some degree.	19
C_4H_{10}	2-Methyl- propane- $2-H^2$	H_2SO_4	25	5 hrs.	No exchange of deuterium with acid.	22
C_4H_{10}	2-Methyl- propane- $1-H^2$	H_2SO_4	25	50% exchange in 3 hours; rate increased by addition of 0.1% 2-methylpropene.	22
C_3H_{10} C_3H_{12}	Cyclopentane 2,2-Dimethyl- propane	1.2 mmoles	95% $H_2^2SO_4$ H_2^2O	2 ml. Silica- alumina	25 340	40 min. 1 hr.	No exchange. 6.5 mole % deuteration.	37 19
C_3H_{12}	2,2-Dimethyl- propane	1.94 mm.	H_2^2	19.4 mm.	Pd film	148	30 min.	H^2 distribution: H_2^2 , 40%; H_1^2 , 37.58%; H_3^2 , 16.80%; H_4^2 , 4.70%; H_5^2 , 0.80%; H_6^2 , 0.12%.	40
C_3H_{12}	2,2-Dimethyl- propane	1.94 mm.	H_2^2	19.4 mm.	Ni film	96	H^2 distribution: H_2^2 , 85.0%; H_1^2 , 13.68%; H_3^2 , 1.23%; H_4^2 , 0.09%.	40
C_3H_{12}	2,2-Dimethyl- propane	1.94 mm.	H_2^2	19.4 mm.	W film	0	23 min.	H^2 distribution: H_2^2 , 85.0%; H_1^2 , 13.20%; H_3^2 , 1.40%; H_4^2 , 0.34%; H_5^2 , 0.06%.	40
C_3H_{12}	2,2-Dimethyl- propane	1.94 mm.	H_2^2	19.4 mm.	Rh film	11.4	H^2 distribution: H_2^2 , 85.0%; H_1^2 , 12.57%; H_3^2 , 1.45%; H_4^2 , 0.88%; H_5^2 , 0.10%.	40

(Continued)

TABLE XVI, 16 (Continued)

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C_5H_{12}	2-Methylbutane	H_2O	Pt	90-100	10-20 hrs.	30-40% of the H atoms were exchanged.	23
C_5H_{12}	2-Methylbutane	H_2O	Pt	100	23.3 hrs.	Partition = 0.02.	24
C_5H_{12}	2-Methylbutane	H_2O	Pt	100	23.5 hrs.	Partition = 0.43.	24
C_6H_{12}	Cyclohexane	H_2	Pt	97	11 min.	Half period of exchange.	25
C_6H_{12}	Cyclohexane	H_2O	Pt	10-100	10-20 hrs.	30-40% of H atoms were exchanged.	23
C_6H_{12}	Cyclohexane	H_2	Pt	90	26
C_6H_{12}	Cyclohexane	Pt	98	8.5 min.	Half period of exchange.	27
C_6H_{12}	Cyclohexane	H_2SO_4	room	12 days	Very slight exchange.	28
C_6H_{12}	Cyclohexane	H_2O	silica-alumina	308	1 hr.	10.2 mole % deuteration.	19
C_6H_{12}	Cyclohexane	3.011 g.	H_2O	0.427 g.	$PtO_2 \cdot H_2O$, 0.0810 g.	123-133	3 days	Solvent, 1.636 g. acetic acid; exchange, 0.121 atoms H^2 per molecule.	35
C_6H_{12}	Methylcyclopentane	1.2 mmoles	95% H_2SO_4	2 ml.	25	120 min.	Product species, mole per cent: H_2^2 , 37; H_4^2 , 1.0; H_6^2 , 1.7; H_8^2 , 4.6; H_{10}^2 , 19.7; H_{12}^2 , 14.7; H_{14}^2 , 11.3; H_{16}^2 , 6.7; H_{18}^2 , 2.7.	37
C_6H_{14}	2,2-Dimethylbutane	1.2 mmoles	95% H_2SO_4	2 ml.	25	40 min.	No exchange.	37
C_6H_{14}	2,3-Dimethylbutane	0.6 mmole	95% H_2SO_4	2 ml.	25	40 min.	Product species, mole per cent: H_2^2 , 70.5; H_4^2 , <0.1; H_6^2 , <0.5; H_8^2 , 5.0; H_{10}^2 , 21.5; H_{12}^2 , 3.0.	37
C_6H_{14}	Hexane	3.106 g.	H_2O , 0.427 g.	0.427 g.	$PtO_2 \cdot H_2O$, 0.0805 g.	123-133	3 days	Solvent, 1.635 g. acetic acid; exchange, 0.187 atoms H^2 per molecule.	35

C_6H_{14}	Hexane	H_2SO_4	room	15 days	Statistical exchange number, 1.82.	28
C_6H_{14}	Hexane	H_2O	Pt	3 min.	Half period of exchange,	27,25
C_6H_{14}	2-Methyl-pentane	H_2SO_4	13.5 hrs.	96% of equilibrium exchange, if constant is unity.	29
C_6H_{14}	2-Methyl-pentane	1.2 mmoles	95% H_2SO_4	2 ml.	40 min.	Product species, mole per cent: H_2^3 , 96; H_2^2 , <0.1; H_2^1 , 1.2; H_2^0 , 0.8; H_2^{10} , 1.0; H_2^{12} , <0.1; H_2^{13} , 1.0.	37
C_6H_{14}	3-Methyl-pentane	1.2 mmoles	95% H_2SO_4	2 ml.	40 min.	Product species, mole per cent: H_2^3 , 93; H_2^2 , 0.5; H_2^1 , <0.1; H_2^{10} , 2.7; H_2^{11} , <0.1; H_2^{12} , <0.4; H_2^{13} , 3.3.	37
C_7H_{14}	Ethylcyclo-pentane	0.6 mmole	95% H_2SO_4	2 ml.	120 min.	Product species, mole per cent: H_2^3 , 65; H_2^2 , 0.8; H_2^1 , 0.7; H_2^0 , 1.3; H_2^9 , 4.9; H_2^{10} , 5.7; H_2^{11} , 6.9; H_2^{12} , 7.9; H_2^{13} , 7.2.	37
C_7H_{14}	Methylcyclo-hexane	1.2 mmoles	95% H_2SO_4	2 ml.	120 min.	Product species, mole per cent: H_2^3 , 51; H_2^2 , 0.4; H_2^1 , 0.7; H_2^0 , 2.0; H_2^9 , 7.7; H_2^{10} , 4.3; H_2^{12} , 4.8; H_2^{13} , 5.7; H_2^{11} , 7.0; H_2^{12} , 8.2; H_2^{13} , 8.1.	37
C_7H_{14}	Methylcyclo-hexane	H_2O	silica-alumina	1 hr.	10.3 mole % deuteration.	19
C_7H_{14}	Methylcyclo-hexane	H_2SO_4	room	15 days	Statistical exchange number, 5.43.	28
C_7H_{16}	2,3-Dimethyl-pentane	0.6 mmole	93.6% H_2SO_4	2 ml.	60 min.	Product species, mole per cent: H_2^3 , 97.8; H_2^{12} , 2.1.	37
C_7H_{16}	2,4-Dimethyl-pentane	0.6 mmole	93.6% H_2SO_4	2 ml.	60 min.	Product species, mole per cent: H_2^3 , 96.9; H_2^{12} , 3.4.	37

(Continued)

TABLE XVI, 16 (Continued)

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₇ H ₁₆	3-Ethyl-pentane	0.6 mmole	95% H ₂ SO ₄	2 ml.	25	120 min.	Product species, mole per cent: H ₆ , 96.7; H ₃ , 0.06; H ₁₂ , 1.09; H ₁₃ , 2.11.	37
C ₇ H ₁₆	Heptane	H ₂ SO ₄	room	9 days	Statistical exchange number, 0.06.	28
C ₇ H ₁₆	Heptane	H ₂ O	silica-alumina	280	1 hr.	8.54 mole % deuteration.	19
C ₇ H ₁₆	Heptane	H ₂ O	Ni	160-180	4 hrs.	$k = \frac{(C-H)(OH)}{(C-H)(OH)^2} = 0.13$.	30
C ₇ H ₁₆	Heptane	H ₂	Ni-kieselguhr	122	Atom % deuterium in product, 14.78.	36
C ₇ H ₁₆	Heptane	0.6 mmole	98.6% H ₂ SO ₄	2 ml.	25	240 min.	No exchange.	37
C ₇ H ₁₆	2-Methyl-hexane	1.2 mmoles	95% H ₂ SO ₄	2 ml.	25	120 min.	Product species, mole per cent: H ₂ , 92.5; H ₇ , 0.11; H ₃ , 1.36; H ₃ , 0.68; H ₁₀ , 0.88; H ₁₁ , 0.99; H ₁₂ , 1.59; H ₁₄ , 0.15; H ₁₃ , 1.61. 18% of all hydrogen atoms exchanged.	37
C ₇ H ₁₆	3-Methyl-hexane	95% H ₂ SO ₄	60	5 hrs.	18% of all hydrogen atoms exchanged.	29
C ₇ H ₁₆	3-Methyl-hexane	H ₂	Ni-kieselguhr	130	Atom % deuterium in product, 16.8.	36
C ₇ H ₁₆	2,2,3-Tri-methyl-butane	H ₂	Ni-kieselguhr	119	Atom % deuterium in product, 1.91.	36
C ₇ H ₁₆	2,2,3-Tri-methyl-butane	0.6 mmole	93.6% H ₂ SO ₄	2 ml.	25	165 min.	Product species, mole per cent: H ₆ , 95.9; H ₁₃ , 4.0.	37

C_4H_{18}	2,3-Dimethyl- hexane	H_2SO_4	60	24 hrs. No exchange (0.04%).	29
C_6H_{18}	3,3-Dimethyl- hexane	H_2^2	86 Atom % deuterium in product, 0.83.	36
C_6H_{18}	3-Methyl- heptane	$C_2H_5SO_3H^2$	106	50 hrs. 5.72% of all hydrogen exchanged.	29
C_6H_{18}	3-Methyl- heptane	95.6% $H_2^2SO_4$	60	23 hrs. 34.1% of all hydrogen exchanged.	31
C_8H_{18}	Octane	95.6% $H_2^2SO_4$	60	27.5 hrs. 0.3% of all hydrogen exchanged.	29
$C_{10}H_{18}$	Camphane	H^2Cl	0	5 min. Exchange was rapid in $CHCl_3$ solution.	33
$C_{11}H_{26}$	3-Methyl- undecane	$H_2^2SO_4$	60	17.5 min. 27.2% of all hydrogen atoms exchanged.	29

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TABLE XVI, 17
Deuterium Exchange with Alkynes and Alkenes

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C_2H_2	Acetylene	H_2O	$NaOH^2$	room	Acetylene of 97 mole % $C_2H_2^2$ was enriched to 98.7 mole % by 3-4 exchanges.	1
C_2H_2	Acetylene	H_2O	$NaOH^2$	room	Exchange only in alkaline medium	2
C_2H_2	Acetylene	H_2O	$NaOH^2$	0	275 hrs.	Equilibrium $k = 0.37$.	3
C_2H_2	Acetylene	H_2O	$NaOH^2$	25	113 hrs.	Equilibrium $k = 0.45$.	3
C_2H_2	Acetylene	H_2O	$NaOH^2$	100	113 hrs.	Equilibrium $k = 0.51$	3
C_2H_2	Acetylene	51.0%	$C_2H_2^3$	48.0%	$Ni, 2 g.$	119	Final composition of equilibrium mixture: $C_2H_2, 28.5\%$; $C_2H_2H^2, 46.5\%$; $C_2H_2^3, 25.0\%$.	18
C_2H_4	Ethylene	H_2O	nickel	150	2-3 days	Product was a mixture of <i>cis</i> and <i>trans</i> -ethylene-1,2- H_2^3 with ethylene-1,1- H_2^3 , ethylene- H_2^3 and ethylene- H_3 .	4
C_2H_4	Ethylene	H_2^3	Ni wire	156	This was a kinetic study of the exchange and hydrogenation reactions over the temperature range 60-207°. An exchange mechanism was discussed.	5
C_2H_4	Ethylene	0.06 g.	H_2O	0.12 g.	0.08 g. Pt black	80	24 hrs.	Partition coefficient = 0.13.	6
C_2H_4	Ethylene	0.06 g.	H_2O	0.10 g.	1.49 g. Ni	80	24 hrs.	Partition coefficient = 0.59.	6
C_2H_4	Ethylene	19.5 ml.	H_2^3	41.4 ml.	Ni	65	<15 min.	Exchange, 10.3%. Kinetic study.	7
C_2H_4	Ethylene	1 mole	H_2^3	2 moles	Ni wire	90	Exchange was more rapid than addition. Kinetic study.	8

(Continued)

TABLE XVI, 17 (Continued)

Formula	Compound	Conc. or amount	Isotope source	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₂ H ₄	Ethylene	20 mm.	H ²	24 mm.	Ni wire	155	Approximately 65% exchange and 35% hydrogenation to ethane. Ratio of exchange to hydrogenation increases with temperature. Partition coefficient = 0.93	9
C ₂ H ₄	Ethylene	0.062 mole	H ₂ O	0.083 mole	1.5 g. Pd	140	118 hrs.	Exchange, 93%. The ratio of exchange to hydrogenation at 235° was 13.3	10
C ₂ H ₄	Ethylene	22 mm.	H ₂	39.5 mm.	Pt foil	235	Equilibrium was established. A mechanism study.	11
C ₂ H ₄	Ethylene	1 mole	H ₂	1 mole	Ni	97	Equilibrium established. Distribution coefficient between water and ethylene = 1.7.	12
C ₂ H ₄	Ethylene	35 ml.	H ₂ O	100 mg.	1.5 g. Ni	80	24 hrs.	Final composition of equilibrium mixture: C ₂ H ₂ , 5.3%; C ₂ HH ² , 20.4%; C ₂ H ₂ , 24.8%; CH ₃ C≡CH, 19.5%; CH ₃ C≡CH ² , 30.0%.	13
C ₃ H ₄	Propyne	49.5%	C ₂ H ₂ C ₂ HH ²	49.5% 1.0%	Ni, 2 g.	62	Equilibrium established, exchange at all the hydrogen atoms.	18
C ₃ H ₅	Propene	1 mole	H ₂	1 mole	Ni	97	Exchange, 3.4%	12
C ₃ H ₅ C ₄ H ₈	Propene 2-Butene	19.4 ml.	H ₂ H ₂	38.5 ml.	Cu Ni	0 76-126	<15 min.	Hydrogenation, exchange and double bond migration occur simultaneously, starting with 1-butene.	7 14
C ₄ H ₈	2-Butene	1 mole	H ₂	1 mole	Ni wire	93-130	Hydrogenation, exchange and double bond migration occur simultaneously, starting with 1-butene.	15

C_4H_8	2-Butene	1.36 mmoles	H_2^2O	silica- alumina	20	22.6% deuteration.	16
C_4H_8	1- and 2- Butene	0.09 mole	H_2^2O	0.08 mole	8 g. Ni	110	166 hrs.	Exchange, 88% of equilibrium.	10
C_4H_8	1- and 2- Butene	0.021 mole	H_2^2O	0.058 mole	2 g. Pd	110	235 hrs.	Exchange, 57% of equilibrium.	10
C_4H_8	2-Butene	1 mole	H_2^2	1 mole	Ni	133	19 min.	Exchange, double bond migration and hydrogenation all occur, starting with 1-butene. Ex- change of all H atoms.	12
C_4H_8	2-Methyl- propene	1 mole	H_2^2	1 mole	Ni	97	Exchange to equilibrium; also hy- drogenation. Exchange of all H atoms.	12
C_5H_{10}	2-Methyl- 2-butene	1 mole	H_2^2	1 mole	Ni	97	Exchange to equilibrium; also hy- drogenation. Exchange of all H atoms.	12
C_5H_{10}	1-Pentene	2.567 g.	H_2^2O	0.429 g.	0.0810 g. $PtO_2 \cdot H_2O$	123-133	3 days	In 1.641 g. acetic acid solvent, 0.98 H^2 atoms per molecule ex- changed.	17
C_6H_8	1,4-Cyclo- hexadiene	2.841 g.	H_2^2O	0.434 g.	0.0810 g. $PtO_2 \cdot H_2O$	123-133	3 days	In 1.64 g. acetic acid solvent, 2.7 atoms H^2 per molecule exchanged.	17
C_6H_8	1,3-Cyclo- hexadiene	2.855 g.	H_2^2O	0.437 g.	0.0810 g. $PtO_2 \cdot H_2O$	123-133	3 days	Solvent, 1.651 g. acetic acid; 0.64 atoms H^2 per molecule exchanged.	17
C_6H_{10}	Cyclohexene	2.973 g.	H_2^2O	0.439 g.	0.0810 g. $PtO_2 \cdot H_2O$	123-133	3 days	Solvent, 1.645 g. acetic acid; ex- change, 0.64 atoms H^2 per molecule.	17
C_8H_{16}	1-Octene	3.977 g.	H_2^2O	0.433 g.	0.0810 g. $PtO_2 \cdot H_2O$	123-133	3 days	Solvent, 1.651 g. acetic acid; ex- change, 1.13 atoms H^2 per molecule.	17
C_8H_{16}	2-Octene	3.976 g.	H_2^2O	0.431 g.	0.0810 g. $PtO_2 \cdot H_2O$	123-133	3 days	Solvent, 1.644 g. acetic acid; ex- change 1.01 atoms H^2 per molecule.	17

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TABLE XVI, 18

Deuterium Exchange with Amides

Formula	Compound	Isotope source	Temp., °C.	Time	Notes	Ref.
$\text{CH}_4\text{N}_2\text{O}$	Urea	H_2O	Determination of active hydrogen by exchange. The dry substance was weighed before and after exchange. Urea exchanges all 4 H atoms.	1
$\text{CH}_4\text{N}_2\text{O}$	Urea	H_2O	Repeated recrystallization from water- H_2^2 gave urea with 99.6% deuterium.	2
$\text{CH}_4\text{N}_2\text{O}$	Urea	H_2O	The observed partition coefficient was 0.98 ± 0.01 .	3
$\text{CH}_4\text{N}_2\text{O}$	Urea	H_2O	The partition coefficient was 1.00 ± 0.05 .	4
$\text{CH}_4\text{N}_2\text{O}$	Urea	H_2O	Exchange equilibrium constant measured.	5
$\text{C}_2\text{H}_4\text{ClNO}$	2-Chloroacetamide	H_2O	Exchange of amide hydrogens.	7
$\text{C}_2\text{H}_5\text{NO}$	Acetamide	H_2O	The partition coefficient was 1.02 ± 0.02 .	3
$\text{C}_2\text{H}_5\text{NO}$	Acetamide	H_2O	The partition coefficient was 1.02 ± 0.02 .	4
$\text{C}_4\text{H}_5\text{NO}_2$	Succinimide	$\text{C}_2\text{H}_5\text{OH}^2$	110	25 hrs.	Exchange of 1 H atom.	6

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TABLE XVI, 19
Deuterium Exchange with Amines

Formula	Compound	Moles	Isotope source	Moles	Catalyst	Temp., °C.	Time	Exchange number	Notes	Ref.
CH ₅ N	Methylamine	2.59 mm.	H ₂	12.9 mm.	Pd film	30	Product: methylamine (70.0%), methylamine-N-H ₂ (27.4%), methylamine-N-H ₃ (2.6%).	19
CH ₅ N	Methylamine	2.59 mm.	H ₂	12.9 mm.	Ni film	52	Product: methylamine (70.0%), methylamine-N-H ₂ (26.7%), methylamine-N-H ₃ (3.0%), methylamine-N, 1-H ₃ (0.3%), methylamine-N, 1, -1-H ₂ (0.1%).	19
CH ₅ N	Methylamine	2.59 mm.	H ₂	12.9 mm.	W film	102	Product: methylamine (80.0%), methylamine-N-H ₂ (17.8%), methylamine-N-H ₃ (1.6%), methylamine-N, 1-H ₃ (0.6%).	19
CH ₅ N	Methylamine	2.59 mm.	H ₂	12.9 mm.	Fe film	79	Product: methylamine (80.0%), methylamine-N-H ₂ (14.5%), methylamine-N-H ₃ (3.3%), methylamine-N, 1-H ₃ (2.1%), methylamine-N, -1-H ₂ (0.1%).	19
CH ₅ N	Methylamine	2.59 mm.	H ₂	12.9 mm.	Pt film	-19.6	k = 4.3%/min. Poisoning of the catalyst occurred at all temperatures.	19
CH ₆ ClN	Methylamine · HCl	...	H ₂ O	room	...	3	Methylamine-N-H ₂ · H ² Cl.	1
CH ₆ ClN	Methylamine · HCl	0.002	H ₂ O	0.035	...	room	30 min.	3.1	Methylamine-N-H ₂ · H ² Cl.	2

C_2H_7N	Dimethylamine	1.94 mm.	H_2^2	12.9 mm.	Pd film	0	Product: dimethylamine (90.0%), dimethylamine- $N-H^2$ (10.0%).	19
C_2H_7N	Dimethylamine	1.94 mm.	H_2^2	12.9 mm.	Ni film	70	Product: dimethylamine (57.5%), dimethylamine- $N-H^2$ (39%), dimethylamine- $N,1-H_2^2$ (1.24%), H_2^2 -dimethylamine (0.97%), H_2^2 - (0.68%), H_2^2 - (0.33%), H_2^2 - (0.29%), H_2^2 - (0.20%).	19
C_2H_7N	Dimethylamine	1.94 mm.	H_2^2	12.9 mm.	W film	90	Product: dimethylamine (80.0%), dimethylamine- $N-H^2$ (15.8%), dimethylamine- $N,1-H_2^2$ (2.2%), H_2^2 -dimethyl- amine (1.0%), H_2^2 - (0.35%), H_2^2 - (0.21%), H_2^2 - (0.42%).	19
C_2H_7N	Dimethylamine	1.94 mm.	H_2^2	12.9 mm.	Fe film	79	Product: dimethylamine (80%), dimethylamine- $N-H^2$ (15.6%), dimethylamine- $N,1-H_2^2$ (2.3%), H_2^2 -dimethylamine (1.2%), H_2^2 - (0.6%), H_2^2 - (0.25%), H_2^2 - (0.07%).	19
C_2H_7N	Ethylamine	H_2O	Equilibrated, dried and distilled.	20
C_2H_5ClN	Dimethyl- amine $\cdot HCl$	0.002	H_2O	0.036	room min.	30	2.15	Dimethylamine- $N-H^2 \cdot H^2Cl$	2

(Continued)

TABLE XVI, 19 (Continued)

Formula	Compound	Moles mm.	Isotope source	Moles mm.	Catalyst	Temp., °C.	Time	Exchange number	Notes	Ref.
C_3H_9N	Trimethylamine	1.29 mm.	H_2^3	12.9 mm.	W film	45	Product: trimethylamine (48%), trimethylamine- H_2^3 (26.9%), H_2^3 -trimethylamine (11.8%), H_2^3 - (5.3%), H_2^3 - (2.5%), H_2^3 - (1.6%), H_2^3 - (1.0%), H_2^3 - (0.8%), H_2^3 - (0.8%), H_2^3 - (1.3%).	19
C_3H_9N	Trimethylamine	1.29 mm.	H_2^3	12.9 mm.	Fe film	128	Product: trimethylamine (50.0%), trimethylamine- H_2^3 (30.5%), H_2^3 -trimethylamine (11.5%), H_2^3 - (5.2%), H_2^3 - (1.9%), H_2^3 - (0.6%), H_2^3 - (0.2%), H_2^3 - (0.1%).	19
C_3H_9N	Trimethylamine	1.29 mm.	H_2^3	12.9 mm.	Pd film	91	Product: trimethylamine (60.0%), trimethylamine- H_2^3 (28.4%), H_2^3 -trimethylamine (8.1%), H_2^3 - (2.8%), H_2^3 - (0.6%), H_2^3 - (0.05%).	19
C_6H_7N	Aniline	0.110 mole	$H_2^{18}O$	1 g.	...	40	24 hrs.	...	Partition coefficient, 1.11. 21,22 Exchange of amino H's.	21,22
C_6H_8ClN	Aniline · HCl	0.2	$H_2^{18}O$	100	30 hrs.	...	Labile deuterium removed by exchange with water. Deu- terium in nucleus is in 2 and 4 positions.	3
C_6H_8ClN	Aniline · HCl	...	$H_2^{18}O$	room	About 90% deuterium in amino group.	4

C_6H_8ClN	Aniline \cdot HCl	0.1	H_2O	0.89	100	24 hrs.	5	Product, after repeated equilibration with H_2O , is aniline- $N-H_2-2,4,6-H_3 \cdot H^2Cl$.
C_6H_8ClN	Aniline \cdot HCl	0.0174	H_2O	0.0558	0.5 N KOH	100	105 min.	5.43	6	Rearrangement follows exchange, part of deuterium is on nucleus.
C_6H_8ClN	Aniline \cdot HCl	2.25 g.	H_2O	1 g.	100	6 hrs.	6.18	23	Reaction rate was independent of aniline conc.
C_6H_8ClN	Aniline \cdot HCl	2.25 g.	H_2O	1 g.	3 N HCl	100	6 hrs.	6.02	23	Reaction rate was independent of acid conc.
C_6H_8ClN	Aniline \cdot HCl	0.0174	H_2O	0.0554	100	25 hrs.	6.41	7	Rearrangement follows exchange, part of deuterium is on nucleus.
C_6H_8ClN	Aniline \cdot HCl	H_2O	80	3 hrs.	3.1	8	Exchange number is for nuclear hydrogen atoms only.
C_6H_8ClN	Aniline \cdot HCl	194	3 hrs.	2.2	9	This is an intramolecular rearrangement in solid phase. Deuterium attached to nitrogen exchanges with o - and p -H of benzene ring.
C_6H_8ClN	Aniline \cdot HCl	200	1 hr.	3.0	9	Same as above. The product is aniline- $N-H_2-2,4,6-H_3 \cdot H^2Cl$.
C_7H_9N C_7H_9N	o -Toluidine o -Toluidine	H_2O H_2O	alkali acid	170 170 97 hrs.	Complete Complete	10 10	Exchange in amino group only. Exchange in amino group and in o - and p - positions of ring.
C_7H_9N	o -Toluidine	H_2O	11	Exchanges two H atoms per mole.
C_7H_9N	m -Toluidine	H_2O	alkali	170	Complete	10	Exchange in amino group only.

(Continued)

TABLE XVI, 19 (Continued)

Formula	Compound	Moles	Isotope source	Moles	Catalyst	Temp., °C.	Time	Exchange number	Notes	Ref.
C_7H_9N	<i>m</i> -Toluidine	H_2O	acid	170	>97 hrs.	Complete	Exchange in amino group and in <i>o</i> - and <i>p</i> -position of ring.	10
C_7H_9N	<i>p</i> -Toluidine	H_2O	alkali	170	Complete	Exchange in amino group only.	10
C_7H_9N	<i>p</i> -Toluidine	H_2O	acid	170	18 hrs.	Complete	Exchange in amino group and in <i>o</i> - and <i>p</i> -positions of ring.	10
C_7H_9NO	<i>o</i> -Anisidine	H_2O	Exchanges two H atoms/molecule.	11
$C_7H_{10}ClN$	<i>p</i> -Toluidine • HCl	180	Intramolecular exchange between amino deuterium and 2 H atoms of ring. Solid phase.	12
$C_7H_{10}ClN$	<i>p</i> -Toluidine • HCl	243	Intramolecular exchange rapid above 243°, liquid phase. Amino deuterium exchanges with 4 H atoms of ring.	12
$C_7H_{17}N$	Heptylamine	Ethylamine- $N-H_3^2$	-11	2-3 sec.	Exchange rapid.	20
$C_8H_{10}BrN$	<i>m</i> -Bromo- <i>N,N</i> -dimethylaniline	0.0068	ethanol- H^2	0.0858	110	120 hrs.	1.21	Number of H atoms exchanged.	15
$C_8H_{10}BrN$	<i>m</i> -Bromo- <i>N,N</i> -dimethylaniline	0.0137	ethanol- H^2	0.0858	2 mg. NaOH	110	96 hrs.	0.08	Number of H atoms exchanged.	15
$C_8H_{10}BrN$	<i>m</i> -Bromo- <i>N,N</i> -dimethylaniline	0.0137	ethanol- H^2	0.0858	100 mg. H_2SO_4	110	96 hrs.	2.41	Number of H atoms exchanged.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>m</i> -nitroaniline	0.012	ethanol- H^2	0.0858	2 mg. NaOH	110	96 hrs.	0.07	Number of H atoms exchanged.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>m</i> -nitroaniline	0.012	ethanol- H^2	0.0858	100 mg. H_2SO_4	110	98 hrs.	2.08	Number of H atoms exchanged.	15

$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>o</i> -nitroaniline	0.0276	ethanol- H^2	0.0858	110	111 hrs.	No exchange apparent.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>o</i> -nitroaniline	0.0276	ethanol- H^2	0.0858	2 mg. NaOH	110	98 hrs.	No exchange apparent.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>o</i> -nitroaniline	0.0276	ethanol- H^2	0.0858	140 mg. H_2SO_4	110	99 hrs.	0.24	Number of H atoms exchanged.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>o</i> -nitroaniline	0.0091	ethanol- H^2	0.0858	110	101 hrs.	0.03	Number of H atoms exchanged.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>p</i> -nitroaniline	0.0097	ethanol- H^2	0.0858	2 mg. NaOH	110	97 hrs.	0.06	Number of H atoms exchanged.	15
$C_8H_{10}N_2O_2$	<i>N,N</i> -Dimethyl- <i>p</i> -nitroaniline	0.012	ethanol- H^2	0.0858	100 mg. H_2SO_4	110	96 hrs.	1.62	Number of H atoms exchanged.	15
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.24	H_2O	0.90	0.2 mol. HCl	room	48 days	13
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.041	ethanol- H^2	0.085	110	96 hrs.	~0.5	Based on exchange of 1 H atom. Alcohol was 9.1 mole % $C_2H_5OH^2$.	14
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.041	ethanol- H^2	0.085	0.02 <i>M</i> NaOH	110	96 hrs.	~0.4	Based on exchange of 1 H atom. Alcohol was 9.1 mole % $C_2H_5OH^2$.	14
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.041	ethanol- H^2	0.085	0.01 <i>M</i> H_2SO_4	110	96 hrs.	~3	Alcohol was 9.1 mole % $C_2H_5OH^2$.	14
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.0393	ethanol- H^2	0.0858	110	96 hrs.	0.06	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.0393	ethanol- H^2	0.0858	2 mg. NaOH	110	137 hrs.	0.04	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_8H_{11}N$	<i>N,N</i> -Dimethyl-aniline	0.0393	ethanol- H^2	0.0858	140 mg. H_2SO_4	110	96 hrs.	2.55	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_8H_{11}N$	<i>D</i> - α -Methylbenzylamine	H_2O	Two amino H atoms exchanged.	16
$C_{10}H_9N$	Quinaldine	0.0349	ethanol- H^2	0.085	110	60 hrs.	~2	Alcohol was 9.1 mole % $C_2H_5OH^2$.	14

(Continued)

TABLE XVI, 19 (Continued)

Formula	Compound	Moles	Isotope source	Moles	Catalyst	Temp., °C.	Time hrs.	Exchange number	Notes	Ref.
$C_{10}H_9N$	Quinaldine	0.0363	ethanol- H^2	0.085	110	96 hrs.	0.67	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_{10}H_9N$	Quinaldine	0.0363	ethanol- H^2	0.085	25	24 hrs.	0.29	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_{12}H_{11}N$	Diphenylamine	0.0296	ethanol- H^2	0.0858	110	115 hrs.	1.13	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_{12}H_{11}N$	Diphenylamine	0.0296	ethanol- H^2	0.0858	2 mg. NaOH	110	115 hrs.	1.14	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_{12}H_{11}N$	Diphenylamine	0.0296	ethanol- H^2	0.0858	140 mg. H_2SO_4	110	96 hrs.	5.98	Alcohol was 20 mole % $C_2H_5OH^2$.	15
$C_{12}H_{12}N_2$	Benzidine	1 g.	H_2^2O	1 ml.	0.01 ml. 36 N H_2SO_4	100	168 hrs.	1.72	17
$C_{12}H_{12}N_2$	Benzidine	H_2^2O	base	170	100 hrs.	...	Fast exchange with $N-H$. Slow exchange with <i>ortho</i> H 's, 30% of equilibrium.	10
$C_{12}H_{12}N_2$	Benzidine	1 g.	H_2^2O	acid	170	60 hrs.	...	Fast exchange with $N-H$. Slow exchange with <i>ortho</i> H 's, 30% of equilibrium.	10
$C_{13}H_{11}N$	9-Fluorenamine	0.0083	ethanol- H^2	110	96 hrs.	...	Decomposition.	15
$C_{13}H_{11}N$	9-Fluorenamine	0.011	ethanol- H^2	2 mg. NaOH	110	115 hrs.	...	Decomposition.	15
$C_{13}H_{11}N$	9-Fluorenamine	0.0083	ethanol- H^2	70 mg. H_2SO_4	110	97 hrs.	...	Decomposition.	15
$C_{14}H_{11}N$	2-Methylbenzo- [b]-quinoline	0.025	ethanol- H^2	0.085	110	104 hrs.	1	Alcohol was 9.1 mole % $C_2H_5OH^2$.	14

$C_{14}H_{11}N$	2-Methylbenzo- [b]-quinoline	0.025	ethanol- H^2	0.085	0.02 M NaOH	110	108 hrs.	2	Alcohol was 9.1 mole % $C_2H_5OH^2$.	14
$C_{15}H_{14}NO$	N-Benzylidene- β -methoxy- benzylamine	ethanol- H^2	0.1 N NaOC ₂ H ₅	74	1 hr.	14.6%	Alcohol was 94.4 mole % $C_2H_5OH^2$. Exchange calc'd. on basis of 1 H atom per molecule.	18
$C_{15}H_{14}NO$	N-(β -Methoxy- benzylidene)- benzylamine	ethanol- H^2	0.1 N NaOC ₂ H ₅	74	1 hr.	13.3%	Alcohol was 94.4 mole % $C_2H_5OH^2$. Exchange calc'd. on basis of 1 H atom per molecule.	18
$C_{15}H_{15}N$	N,N-Dimethyl-9- fluorenamine	0.0095	ethanol- H^2	0.0858	1 mg. NaOH	110	97 hrs.	Decomposition.	15
$C_{15}H_{15}N$	N,N-Dimethyl-9- fluorenamine	0.0095	ethanol- H^2	0.0858	70 mg. H_2SO_4	110	96 hrs.	Decomposition.	15

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TABLE XVI, 20

Deuterium Exchange with Amino Acids

Formula	Amino acid	Weight of acid	Isotope source	Volume	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_2H_5NO_2$	Glycine	3.0 g.	H_2O	5 ml.	5 ml. conc. HCl	96	100 hrs.	The liquid was distilled off <i>in vacuo</i> , the residue was dissolved in 10 ml. of water and evaporated. After process was repeated 4 times, product contained 0.92 ± 0.02 atom % deuterium.	1
$C_2H_5NO_2$	Glycine	6.0 g.	H_2O	10 ml.	1 ml. conc. HCl.	130	21 days	Glycine- N^{15} was treated in an evacuated, sealed tube. Product had 28 atom % deuterium.	2
$C_2H_5NO_2$	Glycine	H_2O	Exchange in amino group is reversible with an exchange coefficient of 1.00 ± 0.05 .	3
$C_3H_5NO_2$	Glycine	H_2O	Distribution ratio $\alpha = (H^2/H \text{ in water}) / (H^2/H \text{ in solute})$ was approximately unity.	4
$C_3H_7NO_2$	Alanine	H_2SO_4 , 95-99% acid	100	24 hrs.	Product contained 1.30 atom % deuterium which was not removed by boiling with 20% sulfuric acid for 24 hours.	5
$C_3H_7NO_2$	Alanine	1.5 g.	H_2SO_4 , 91.6% acid	3 ml.	100	48 hrs.	The solution was diluted with 400 ml. of water, neutralized with barium hydroxide and filtered. The product was repeatedly precipitated from water with alcohol.	1

(Continued)

Formula	Amino acid	Weight of acid	Isotope source	Volume	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_5H_9NO_3$	L(-)-Proline	0.98 g.	$H_2^{18}O$, (50%)	10 ml.	10 ml. conc. HCl	reflux	20 hrs.	The water- H_2^{18} was distilled off <i>in vacuo</i> , the residue was dissolved in 30 ml. of water and evaporated to dryness. After this procedure was repeated 6 times, the residue was taken up in 30 ml. of water, adjusted to pH 6 with $NaHCO_3$ and evaporated to dryness. The proline was extracted from the residue with hot alcohol.	1
$C_5H_9NO_3$	Hydroxy-proline	8.386 mg.	$H_2^{18}O$, (99.5%)	0.25 ml.	Micro method of determining active hydrogen by increase in weight of compound. Apparently 3 H atoms exchanged.	6
$C_5H_9NO_4$	L(+)-Glutamic	4.0 g.	$H_2^{18}O$, (56%)	3.5 ml.	3.5 ml. conc. HCl	98	5 days	The solution was evaporated to dryness <i>in vacuo</i> , then dissolved in water and evaporated to dryness 3 times. The final residue in 50 ml. of water was neutralized to Congo red with NH_4OH . The product, which was collected, washed and recrystallized twice from hot water, contained 2.01 ± 0.02 atom % deuterium.	1

$C_6H_{12}N_2O_4S_2$	L(-)- Cystine	6.0 g.	H_2O , (56%)	9 ml.	9 ml. conc. HCl	98	5 days	1
Some decomposition. Solution was diluted with water, treated with charcoal and filtered. The filtrate was evaporated to dryness under vacuum, dissolved in water and again evaporated to dryness. After this was repeated 4 times, the residue was dissolved in 150 ml. of water, again decolorized and neutralized with NH_4OH . The crystals were recrystallized twice by dissolution in dilute hydrochloric acid and neutralization. The product, 4.08 g., contained 1.51 ± 0.03 atom % deuterium.								
$C_6H_{13}NO_2$	D-Leucine	H_2SO_4 , (96-99%)	100	24 hrs.	5
$C_6H_{13}NO_2$	Leucine	1.5 g.	H_2SO_4 , 91.6%	3 ml.	100	48 hrs.	1
$C_6H_{14}N_2O_2$	L(+)- Lysine	2 g. of hydro- chloride	H_2O , (56%)	1.5 ml.	1.5 ml. conc. HCl	98	5 days	1
$C_8H_{10}NO_2$	2-Phenyl- glycine	7.5 g.	H_2O , (22.7 atom %)	25.0 ml.	4 g. NaOH	115	15 hrs.	7
$C_9H_{11}NO_2$	Phenyl- alanine	1.0 g.	H_2O , (50%)	10 ml.	10 ml. conc. HCl	reflux	18 hrs.	1

The product, 6.1 g., contained 2.28 atom % H^2 in the α -position.

Exchange negligible, deuterium content 0.04 ± 0.03 atom %.

(Continued)

TABLE XVI, 20 (Continued)

Formula	Amino acid	Weight of acid	Isotope source	Volume	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₉ H ₁₁ NO ₃	L(-)- Tyrosine	5.5 g.	H ₂ O, (50%)	10 ml.	10 ml. conc. HCl	reflux	18 hrs.	The solvent was distilled off <i>in vacuo</i> . The residue was dissolved in 1 liter of dilute hydrochloric acid, decolorized with charcoal and filtered. The filtrate was adjusted to pH 6 with NaOH and the tyrosine was collected. Dissolution of the product in dilute acid and precipitation at pH 6 was repeated 3 times. The product, 2.08 g., contained 0.91 ± 0.05 atom % H ² .	1

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TABLE XVI, 21
Deuterium Exchange with Aromatic Hydrocarbons

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
C_6H_6	Benzene	H^2Cl	$AlCl_3$	50	3 hrs.	92% of deuterium in H^2Cl exchanged with benzene.	1
C_6H_6	Benzene	H^2Cl	$AlCl_3$	The product was indicated to be $C_6H_6^2$.	2
C_6H_6	Benzene	H_2^2O	Ni	200	2 hrs.	Partition coefficient after 2,6 and 12 hours was 1.05.	3
C_6H_6	Benzene	$H_2^2SO_4$...	room	24 hrs.	Exchange significant.	4
C_6H_6	Benzene	H_2^2	Pt or Ni	20	...	Rate Study.	5
C_6H_6	Benzene	H_2^2O	silica-alumina	110	1 hr.	25.5% mole % deuteration.	6
C_6H_6	Benzene	H_2^2	Pt or Cr_2O_3	100	...	Exchange and hydrogenation.	7
C_6H_6	Benzene	H_2^2O	Pt	room	...	Slight exchange under influence of Tesla coil high frequency discharge.	8
C_6H_6	Benzene	H_2^2	Pt-black or Ni	19	...	Rate study.	9
C_6H_6	Benzene	H_2^2O	Ni	200	...	Exchange at equilibrium in 2 hours.	10
C_6H_6	Benzene	H_2^2	platinized Pt foil	17	...	Comparison of <i>para</i> -hydrogen conversion and exchange.	11
C_6H_6	Benzene	H_2^2	platinized Pt foil	Comparison of hydrogenation and exchange rates.	12
C_6H_6	Benzene	54.9 mole % $H_2^2SO_4$...	room	108 hrs.	Exchange to equilibrium; 10% of benzene sulfonated.	13
C_6H_6	Benzene	64.8 mole % $H_2^2SeO_4$...	room	144 hrs.	Exchange to about 28% of equilibrium.	13
C_6H_6	Benzene, 2.771 g.	H_2^2O , 0.434 g.	$PrO_2 \cdot H_2O$, 0.0810 g.	123-133	3 days	Solvent, 1.640 g. of acetic acid; 1.01 atoms H per molecule exchanged.	20

(Continued)

TABLE XVI, 21 (Continued)

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₆ H ₆	Benzene	H ² Br	room	160- 1550 hrs.	Exchange number = $0.2-1.4$; $k = 4 \times 10^{-8}$ sec. ⁻¹ .	23
C ₆ H ₆	Benzene	H ₂ ² SO ₄	room	150 hrs.	Product was: C ₆ H ₆ , 62.8%; C ₆ H ₅ H ² , 29.2%; C ₆ H ₄ H ₂ ² , 6.8%; C ₆ H ₃ H ₃ ² , 1.06%; C ₆ H ₂ H ₄ ² , 0.13%; C ₆ H ₂ H ₄ ² , 0.01%.	22
C ₇ H ₈	Toluene	H ² Br	25	2 hrs.	Exchange number = $1.4-1.7$; $k = 3 \times 10^{-4}$ sec. ⁻¹ for <i>p</i> -H, 5×10^{-5} sec. ⁻¹ for <i>o</i> -H and 2.5×10^{-7} sec. ⁻¹ for <i>m</i> -H. Same values apply for ethylbenzene, isopropylbenzene and <i>t</i> -butylbenzene.	23
C ₈ H ₈	Styrene	H ² Cl	8.5 N HCl	150	10 hrs.	Exchange takes place with monomer before polymerization.	14
C ₈ H ₈	Styrene	C ₂ H ₅ OH ²	110	97 hrs.	Exchange number, 0.62 (H atoms per molecule).	15
C ₈ H ₈	Styrene	C ₂ H ₅ OH ²	130	22 hrs.	No exchange, but C ₂ H ₅ OH ² occluded in polymer.	16
C ₈ H ₁₀	<i>m</i> -Xylene	H ² Br	25	30 min.	Exchanged 4 H atoms on nucleus, none in methyl groups.	23
C ₉ H ₈	Indene	H ₂ O	0.3 N KOH	100	28 hrs.	Exchange equivalent, 2.42.	21
C ₉ H ₁₂	Mesitylene	C ₂ H ₅ OH ²	alkali	150	30 hrs.	Exchange to 15% of equilibrium.	17
C ₉ H ₁₂	Mesitylene	H ² Br	25	30 min.	Exchanged 3 H atoms on nucleus, none in methyl groups.	23
C ₁₀ H ₈	Naphthalene	H ² Br	25	30 min.	Exchange number = 4.5; $k = 3 \times 10^{-3}$ sec. ⁻¹ for α -H; $k = 7 \times 10^{-5}$ sec. ⁻¹ for β -H.	23
C ₁₀ H ₁₂	1,2,3,4-Tetrahydronaphthalene	H ² Br	25	30 min.	Exchanged all H atoms on aromatic nucleus.	23
C ₁₀ H ₁₄	Durene	H ² Br	25	30 min.	Exchanged 2 H atoms on nucleus, none in methyl groups.	23

$C_{11}H_{14}O$ 2', 4', 6'-Trimethyl- acetophenone	$C_2H_5OH^2$	2 mg, NaOH	110	97 hrs.	Exchange number, 2.49 H atoms per molecule.	15
$C_{11}H_{16}$ Pentamethyl- benzene	H^2Br	25	30 min.	Exchanged 1 H atom on nucleus, none in methyl groups.	23
$C_{11}H_{10}$ Biphenyl	H^2Br	25	1 hr.	Exchange number = 1.2 (in 2-14 days n reached constant value of 6); $k = 2 \times 10^{-4}$ $sec.^{-1}$ for <i>p</i> -H and $2 \times 10^{-5} sec.^{-1}$ for <i>o</i> -H.	23
$C_{12}H_{18}$ Hexamethylbenzene	H^2Br	25	30 min.	No exchange.	23
$C_{13}H_{10}$ Fluorene	$C_2H_5OH^2$	2 mg, NaOH	110	69 hrs.	Exchange number, 1.79 H atoms per molecule.	15
$C_{13}H_{10}$ Fluorene	$C_2H_5OH^2$	0.02 M NaOH	110	2 days	Exchange less than 2 H atoms per molecule.	18
$C_{13}H_{10}$ Fluorene	H^2Br	25	3 hrs.	Exchange number = 5.8.	23
$C_{13}H_{12}$ Diphenylmethane	H^2Br	25	1 hr.	Exchange number = 0.39 (7.3 in 1454 hrs.); $k = 6 \times 10^{-5} sec.^{-1}$ for <i>p</i> -H.	23
$C_{14}H_{10}$ Anthracene	H_2SO_4	room	Product was mixture of deuterated anthracenes.	19
$C_{14}H_{10}$ Anthracene	H^2Br	25	30 min.	Exchange number = 10.	23
$C_{14}H_{10}$ Phenanthrene	H^2Br	25	30 min.	Exchange number = 7.8.	23
$C_{14}H_{14}$ 1,2-Diphenylethane	H^2Br	25	1 hr.	Exchange number = 0.64.	23
$C_{16}H_{10}$ Pyrene	H^2Br	0	30 min.	Exchange number = 10.	23
$C_{16}H_{16}$ Distyrene	H^2Cl	8.5 N HCl	150	10 hrs.	Dimer contains deuterium. Mechanism study.	14
$C_{16}H_{18}$ 1,4-Diphenylbutane	H^2Br	25	1 hr.	Exchange number = 1.7.	23
$C_{18}H_{15}$ <i>p</i> -Terphenyl	H^2Br	25	1 hr.	Exchange number = 2.7 (in 2-14 days n reached a constant value of 10); $k = 7 \times 10^{-4}$ $sec.^{-1}$ for <i>p</i> -H and $6 \times 10^{-4} sec.^{-1}$ for <i>o</i> -H.	23
$C_{19}H_{16}$ Triphenylmethane	H^2Br	25	1 hr.	Exchange number = 0.08 (10.0 in 1454 hrs.); $k = 8 \times 10^{-6} sec.^{-1}$ for <i>p</i> -H.	23
$C_{19}H_{16}$ Triphenylmethane	$C_2H_5OH^2$	140 mg, H_2SO_4	110	105 hrs.	Exchange number 7.93 (H^2 atoms/molecule).	15
$C_{38}H_{30}$ Hexaphenylethane	H_2O	100	6 days	No exchange.	17

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AROMATIC HYDROCARBONS

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TABLE XVI, 22
Deuterium Exchange with Carbohydrates

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_5H_{12}O_4$	Pentae rythritol	H_2O	Water- H_2 was recrystallization solvent.	1
$C_6H_{12}O_5$	2-Deoxyglucose	H_2O , (86%)	...	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of hydrogens exchanged was 3.97.	2
$C_6H_{12}O_6$	Fructose	H_2O	...	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of hydrogen atoms exchanged was 4.81.	2
$C_6H_{12}O_6$	Fructose	H_2O	1 hr.	At equilibrium. Partition coefficient = 1.15.	3
$C_6H_{12}O_6$	Fructose	H_2O	$Ca(OH)_2$	25-55	...	From glucose, base catalyzed rearrangement.	4
$C_6H_{12}O_6$	Fructose	H_2O	$Ca(OH)_2$	35	...	1.68 H atoms bound to carbon exchanged in base catalyzed rearrangement.	6
$C_6H_{12}O_6$	Galactose	H_2O	...	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 4.75.	2
$C_6H_{12}O_6$	Glucose	H_2O	$Ca(OH)_2$	40-55	10 days	Partition coefficient = 2.2. In addition to hydroxyl hydrogen atoms, 2 H atoms bound to carbon were exchanged.	4
$C_6H_{12}O_6$	Glucose	H_2O	base	> 40	...	1 to 2 H atoms exchanged per molecule.	5
$C_6H_{12}O_6$	Glucose	H_2O	acid or base	Equilibrium in less than 1 minute in neutral, acidic or basic medium. Distribution coefficient = 1.15.	3
$C_6H_{12}O_6$	Glucose	H_2O	...	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 5.2.	2
$C_6H_{12}O_6$	Mannose	H_2O	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 4.96.	2
$C_6H_{12}O_6$	Mannose	H_2O	$Ca(OH)_2$	55	10 days	Base catalyzed transformation of glucose. Distribution coefficient = 2.2.	4
$C_6H_{14}O_6$	Mannitol	H_2O	...	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 5.53.	2
$C_6H_{18}O_6$	meso-Inositol	H_2O	Pt	130-150	30 days	Product contained 1.75 atom % of deuterium.	9

(Continued)

TABLE XVI, 22 (Continued)

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_7H_{14}O_6$	α -Methylgalactoside	$H_2^{18}O$	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 3.98.	2
$C_7H_{14}O_6$	α -Methylglucoside	$H_2^{18}O$	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 4.21.	2
$C_7H_{14}O_6$	α -Methylmannoside	$H_2^{18}O$	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 4.04.	2
$C_{10}H_{19}O_6$	Tetramethylglucose	$H_2^{18}O$	room	1 hr.	Equilibrium $k = 0.65$. The estimated number of H atoms exchanged was 1.18.	2
$C_{12}H_{22}O_{11}$	Sucrose	$H_2^{18}O$	Hydroxyl H atoms exchanged.	7
	Cellulose	$H_2^{18}O$	100	13 hrs.	Exchange of 3 H atoms per glucose unit in filter paper cellulose is complete in 13 hours at 100 °; 24 hours at 30 °.	8

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TABLE XVI, 23

Deuterium Exchange with Esters

Formula	Ester	Milli- moles	Isotope source	Milli- moles	Catalyst	Temp., °C.	Time	Ex. No.*	Notes	Ref.
$C_4H_8O_2$	Ethyl acetate	51.1	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	1.3	56% of completion.	1
$C_4H_8O_2$	Ethyl acetate	AcOH 2	150	100 hrs.	20% of completion.	3
$C_5H_{10}O_2$	Ethyl propionate	43.6	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.24	14% of completion.	1
$C_6H_{10}O_3$	Ethyl aceto- acetate	38.4	ethanol- H^2	85.3	room	3 days	2	85% of completion, calculated for ex- change of 2 H atoms, no catalyst.	2
$C_6H_{12}O_2$	Ethyl crotonate	37.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.63	1
$C_6H_{12}O_2$	Ethyl butyrate	37.8	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.11	6.4% of completion.	1
$C_6H_{12}O_2$	Ethyl isobutyrate	37.5	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.02	1.9% of completion.	1
$C_6H_{12}O_3$	Ethyl 2-methoxy- propionate	37.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.03	3.5% of completion.	1
$C_7H_{12}O_2$	Ethyl senecioate	36.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.35	1
$C_7H_{12}O_4$	Ethyl malonate	23.0	ethanol- H^2	85.3	5 mg. sodium salicylate	25	4 hrs.	1.71	85% of completion.	1
$C_7H_{14}O_2$	Ethyl isovalerate	37.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.05	2.9% of completion.	1
$C_8H_{12}O_2$	Ethyl sorbate	37.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.20	1
$C_8H_{14}O_4$	Ethyl methyl- malonate	23.0	ethanol- H^2	85.3	5 mg. sodium salicylate	25	4 hrs.	0.25	25% of completion.	1
$C_9H_{12}O_2$	Ethyl o-toluate	31.5	ethanol- H^2	85.3	40 mg. sodium	110	16 hrs.	0.04	1
$C_9H_{12}O_2$	Ethyl p-toluate	31.2	ethanol- H^2	85.3	40 mg. sodium	110	12 hrs.	0.28	1
$C_9H_{14}O_4$	Ethyl citra- conate	37.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.78	1
$C_9H_{14}O_4$	Ethyl mesa- conate	37.0	ethanol- H^2	85.3	38 mg. sodium	25	4 hrs.	0.07	1

(Continued)

TABLE XVI, 23 (Continued)

Formula	Ester	Milli- moles	Isotope source	Milli- moles	Catalyst	Temp., °C.	Time	Ex. No.*	Notes	Ref.
C ₁₀ H ₁₂ O ₂	Ethyl phenyl- acetate	10.0	ethanol-H ²	85.3	40 mg. sodium	0	5 min.	0.91	48% of completion.	1
C ₁₁ H ₁₄ O ₂	Ethyl hydra- trophate	20.8	ethanol-H ²	85.3	40 mg. sodium	0	5 min.	0.13	13% of completion.	1
C ₁₁ H ₁₄ O ₃	Ethyl 2-methoxy-2- phenylacetate	10.0	ethanol-H ²	85.3	40 mg. sodium	0	5 min.	0.17	17% of completion.	1
C ₁₂ H ₁₆ O ₂	Ethyl 2,4,6-tri- methylbenzoate	15.2	ethanol-H ²	85.3	40 mg. sodium	110	12 hrs.	0.09	1
C ₁₃ H ₁₆ O ₄	Ethyl phenyl- malonate	23.0	ethanol-H ²	85.3	5 mg. sodium salicylate	25	4 hrs.	0.33	30% of completion.	1
C ₁₆ H ₁₆ O ₂	Ethyl diphenyl- acetate	10.0	ethanol-H ²	85.3	40 mg. sodium	0	5 min.	0.25	24% of completion.	1

* Exchange number = hydrogen atoms exchanged, calculated on assumption of statistical distribution of deuterium.

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TABLE XVI, 24, Deuterium Exchange with Ethers

Formula	Ether	Milli- moles	Isotope source	Milli- moles	Catalyst	Temp., °C.	Time, hrs.	Ex. No.*	Notes	Ref.
C ₇ H ₆ O ₂	1,3-Benzodioxole	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	12	1.3	...	1
C ₇ H ₈ O	Anisole	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	46.7	2.42	...	1
C ₇ H ₈ O	Anisole	...	sulfuric acid-H ²	room	6	2.8	Initial concentration of sulfuric acid 54.5 mole %. Six % of anisole was sulfonated.	2
C ₈ H ₈ O ₂	1,4-Benzodioxan	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	120	2.48	...	1
C ₈ H ₁₀ O	2-Methylanisole	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	12	1.41	...	1
C ₈ H ₁₀ O	3-Methylanisole	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	2	2.62	...	1
C ₈ H ₁₀ O	Veratrole	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	48	2.85	...	1
C ₉ H ₁₀ O	2-Methyl-2,3-dihydro-benzofuran	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	18	1.47	...	1
C ₉ H ₁₀ O ₂	3,4-Dihydro-2H-1,5-benzodioxepin	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	156.5	1.49	...	1
C ₉ H ₁₂ O	3,5-Dimethylanisole	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	0.5	2.8	...	1
C ₁₀ H ₁₂ O ₂	2,3,4,5-Tetrahydro-1,6-benzodioxocin	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	24	1.4	...	1
C ₁₄ H ₁₂ O	9-Methoxyfluorene	15.3	ethanol-H ²	...	none	110	95	...	No exchange.	3
C ₁₄ H ₁₂ O	9-Methoxyfluorene	15.3	ethanol-H ²	...	2 mg. NaOH	110	116	...	No exchange.	3
C ₁₄ H ₁₂ O	9-Methoxyfluorene	15.3	ethanol-H ²	...	100 mg. H ₂ SO ₄	110	116	...	Decomposition.	3
C ₁₄ H ₁₂ O ₂	Catechol diisobutyl ether	7	acetic acid-H ²	34.9	3.68 mg. H ₂ SO ₄	90	48	2.85	...	1

*The exchange number, $n = \frac{a}{s} \left(\frac{H_1^2}{H_2^2} - 1 \right)$; in the equation, a is the number of moles of the isotope source, e.g., alcohol-H² or acetic acid-H², s is the number of moles of the compound and H₁² and H₂² represent the initial and final concentrations, respectively, of deuterium in the alcohol or acetic acid.

References to Table XVI, 24, DEUTERIUM EXCHANGE WITH ETHERS

- ¹W. G. Brown, K. E. Wilzbach and W. H. Urry, *Can. J. Research*, 27B, 398 (1949).
²C. K. Ingold, C. G. Raisin and C. L. Wilson, *J. Chem. Soc.*, 1946, 1637.
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TABLE XVI, 25

Deuterium Exchange with Heterocyclics

Formula	Compound	Conc. or Isotope amount	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₄ H ₄ O	Furan	H ₂ O-1N HCl	100	34 hrs.	Exchange equivalent, 2.72. All of hydrogens slowly exchange.	1
C ₄ H ₄ S	Thiophene	H ₂ O-1N HCl	150	30 hrs.	Exchange equivalent, 2.80. All of hydrogens slowly exchange.	1
C ₄ H ₄ S	Thiophene	69% H ₂ SO ₄	40	30 hrs.	Homogeneous product, H ² /H-H ² = 0.967; d ₂ ²⁰ 1.11220; n _D ²⁰ 1.5267; b.p. 82.3° (732.4 mm.).	7
C ₄ H ₅ N	Pyrrrole	0.05 g.	1 g.	1 N HCl	30	40 min.	Exchange equivalent, 3.81. At pH > 1.5, all the hydrogen atoms do not exchange; at pH < 1, acid induced decomposition of the pyrrole is serious. At pH > 2 only N-hydrogen exchanges.	2
C ₄ H ₅ N	Pyrrrole	0.071 mole	0.11 mole	50	5 hrs.	Partition coefficient, 0.88. Only the amino H exchanged.	8,9
C ₅ H ₅ NO	2-Pyridinol	12
C ₅ H ₅ NO	3-Pyridinol	12
C ₅ H ₅ NO	4-Pyridinol	12
C ₅ H ₇ N	1-Methylpyrrole	1 g.	0.4 g.	0.01 N HCl	30	12.7 hrs.	Exchange equivalent, 2.29. No exchange in 10 hours at pH 3 or higher. At pH below 3, H atoms of pyrrole nucleus exchange.	3
C ₆ H ₇ NO	3,5-Dimethyl-4-oxazole-carboxylic acid	0.3 g.	2 g.	20	4 hrs.	The acid was neutralized with 3.5 N sodium hydroxide-H ² solution in water-H ₂ . After exchange, product was precipitated by addition of sulfuric acid-H ₂ . Exchange number 3.39 (theory 3.52); exchange of H atoms in the 5-methyl group through a tautomeric NH form.	4

(Continued)

TABLE XVI, 25 (Continued)

Formula	Compound	Conc. or Isotope amount	Conc. or amount	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_6H_7NO_2S$	2,4-Dimethyl-5-thiazole-carboxylic acid	0.3 g.	H_2O	2 g.	20	4 hrs.	Conditions same as above; exchange in the 2- and 4-methyl groups.	4
C_8H_7N	Indole	3 g.	H_2O	1 g.	3 N HCl	60	5 hrs. Exchange between molten indole and water- H_2 . Exchange number 2.28. Between pH 7 and 0.5, H atom on N exchanges rapidly; between pH 2.5 and 0.5, H atom on β -carbon also exchanges; below pH 0.5, H atom on α -carbon also exchanges.	5
C_8H_7N	Indole	0.0259 mole	H_2O	0.0493 mole	3 N KOH	100	15 hrs. Exchange equivalent, 1.79. In alkaline solution, no step-wise exchange as in acidic media.	11
C_9H_9N	N-Methyl-indole	0.8 g.	H_2O	0.15 ml.	0.1 N HCl	60	5 hrs. Exchange number, 1.09. At pH ≥ 2.5 , no exchange; at pH ≤ 2.5 , rapid exchange with H atoms on β -carbon.	6
C_9H_9N	2-Methyl-indole	0.0041 mole	H_2O	0.0123 mole	0.8 N HCl	60	5 hrs. Exchange equivalent, 2.12. From pH 7.0 to -0.5, two H atoms exchange; H atoms at N and probably β -carbon.	10
C_9H_9N	Skatole	0.0204 mole	H_2O	0.0533 mole	0.8 N HCl	105	5 hrs. Exchange equivalent, 3.18. Exchange of N H atom only between pH 7 and pH 1.	10
$C_9H_9NO_4$	2,6-Dimethyl-3,5-pyridine-dicarboxylic acid	0.3 g.	H_2O	2 g.	20	4 hrs. See procedure for 3,5-dimethyl-4-oxazole-carboxylic acid. Exchange number, 5.5.	4
$C_{10}H_{11}N$	2,3-Dimethyl-indole	1 g.	H_2O	0.25 g.	1.0 N HCl	60	5 hrs. Exchange equivalent, 1.03. At pH values from 7.00 to -0.48 only N H atom exchanges.	10

$C_{11}H_{10}N_4O_2$, 5-Methyl-1-phenyl-3-pyrazolecarboxylic acid	0.3 g.	H ₂ O	3 g.	...	20	4 hrs.	See procedure for 3,5-dimethyl-4-oxazolecarboxylic acid. Exchange number, 4,98. Exchange of H atoms in carboxyl and methyl groups.
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TABLE XVI, 26
Deuterium Exchange with Nitro Compounds

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
CH_3NO_2	Nitromethane	H_2O	In exchange with water- H_2^2 , the equilibrium is toward water- H_2^2 .	1
$\text{C}_6\text{H}_3\text{N}_3\text{O}_6$	1,3,5-Trinitrobenzene	$\text{C}_2\text{H}_5\text{OH}^1$	0.02 M NaOH	110	68 hrs.	Exchange of approximately 1 H atom per molecule.	2
$\text{C}_7\text{H}_6\text{N}_2\text{O}_4$	2,4-Dinitrotoluene	H_2O	alkali	170	68 hrs.	60% exchange.	3
$\text{C}_7\text{H}_7\text{NO}_2$	4-Nitrotoluene	$\text{C}_2\text{H}_5\text{OH}^2$	0.02 M NaOH	110	48 hrs.	Exchange of approximately 2/3 of 1 H atom per molecule.	2
$\text{C}_7\text{H}_7\text{NO}_2$	4-Nitrotoluene	H_2O	alkali	170	68 hrs.	30% exchange in alkaline solution, none in acid.	3
$\text{C}_7\text{H}_7\text{NO}_2$	2-Nitrotoluene	$\text{C}_2\text{H}_5\text{OH}^2$	0.02 M NaOH	110	34 hrs.	Exchange of approximately 1 H atom per molecule.	2

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TABLE XVI, 27
Deuterium Exchange with Onium Compounds

Formula	Compound	Weight, grams	Salt, molarity	Ml. of solution (H ₂ O)	NaOH ¹ , molarity	Time, hours	Temp., °C.	H ² in salt, atom %	Notes	Ref.
C ₃ H ₉ IS	Trimethylsulfonium iodide	0.306	0.30	5.0	0.3076	3	62	98.0	2nd order rate constant at 26.800 ± 0.002 °C., k = 1.17 ± 0.02 × 10 ⁻⁴ .	1
C ₃ H ₉ ISe	Trimethylselenonium iodide	0.226	0.30	3.0	0.2941	3	62	13.2	1
C ₃ H ₉ ITe	Trimethyltelluronium iodide	0.09	0.15	2.0	0.2930	3	62	3.99	1
C ₄ H ₉ OS	Butyl sulfoxide	1.000	(1.24)	5.0	2.22	120	56.4	0.45	The sulfoxide was only partial- ly soluble.	1
C ₄ H ₁₂ ASl	Tetramethylarsonium iodide	0.236	0.30	3.0	0.2947	3	62	7.44	1
C ₄ H ₁₂ IN	Tetramethylammonium iodide	0.603	0.30	10.0	0.2920	358	100	1.13	2nd order rate constant at 100.0 ± 0.1 °C., k = 2.29 ± 0.02 × 10 ⁻³ l. mole sec. ⁻¹	1
C ₄ H ₁₂ IP	Tetramethyl- phosphonium iodide	0.197	0.30	3.0	0.2962	3	62	73.9	2nd order rate constant at 56.4 ± 0.1 °C., k = 4.63 ± 0.08 × 10 ⁻⁴ l. mole sec. ⁻¹	1
C ₄ H ₁₂ ISb	Tetramethylstibonium iodide	0.278	0.30	3.0	0.2881	3	62	0.78	1
C ₇ H ₁₁ IS	Bicyclo[2.2.1]- heptane-1-sulfonium iodide	1.220	1.00	5.0	0.3124	288	56.4	20.1	N of NaOH ² decreased to 0.2078; insoluble oil was present.	1

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TABLE XVI, 28
Deuterium Exchange with Phenols

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
C_6H_5ClO	<i>p</i> -Chlorophenol	H_2O	80% H_2SO_4	20	40 hrs.	A two phase process in which 50 g. of <i>p</i> -chlorophenol in 50 ml. of CCl_4 was shaken with 200 ml. of H_2SO_4 and 12 ml. of H_2O .	20
$C_6H_5NO_3$	<i>o</i> -Nitrophenol	H_2O	3 <i>N</i> KOH	100	1000 hrs.	Exchange number, 3.44 (number of H^2 atoms/molecule).	1
$C_6H_5NO_3$	<i>m</i> -Nitrophenol	H_2O	3 <i>N</i> KOH	100	1000 hrs.	Exchange number, 4.41.	1
$C_6H_5NO_3$	<i>m</i> -Nitrophenol	H_2O	1 <i>N</i> KOH	100	260 hrs.	Exchange number, 2.66.	19
$C_6H_5NO_3$	<i>m</i> -Nitrophenol	H_2O	3 <i>N</i> KOH	100	100 hrs.	Exchange number, 2.92.	2
$C_6H_5NO_3$	<i>p</i> -Nitrophenol	H_2O	3 <i>N</i> KOH	100	1000 hrs.	Exchange number, 3.57.	1
$C_6H_5NO_3$	<i>p</i> -Nitrophenol	H_2O	1 <i>N</i> KOH	100	260 hrs.	Exchange number, 2.52.	19
$C_6H_5NO_3$	<i>p</i> -Nitrophenol	H_2O	3 <i>N</i> KOH	100	100 hrs.	Exchange number, 2.36.	2
$C_6H_5NO_3$	<i>p</i> -Nitrophenol	H_2O	2.93 <i>N</i> KOH	100	100 hrs.	Exchange number, 2.36.	19
$C_6H_5NO_3$	<i>p</i> -Nitrophenol	H_2O	$HClO_4$	100	100 hrs.	Equilibrium was reached in less than 70 hours.	20
	(40 g.)	(4 ml.)	(20 ml.)			Product was recrystallized several times from water and contained two atoms of stably bound deuterium, probably <i>ortho</i> to the OH group.	
C_6H_6O	Phenol	H_2O	NaOH	100	96 hrs.	Proof of exchange in <i>o</i> - and <i>p</i> - positions only (and hydroxyl).	3
C_6H_6O	Phenol	H_2O	NaOH	100	44 hrs.	Exchange number, 3.9.	4
				25	10 hrs.		
C_6H_6O	Phenol	H_2O	Exchange of hydroxyl hydrogen.	5
C_6H_6O	Phenol	H_2O	KOH	122-122.5	10 hrs.	Exchange number, 3.96. Exchange of <i>o</i> -, <i>p</i> - and hydroxyl hydrogens.	6
C_6H_6O	Phenol	H_2O	HCl	100	5 hrs.	Exchange number, 4.05.	7
C_6H_6O	Phenol	H_2O	Exchange of hydroxyl hydrogen.	8

C_6H_6O	Phenol	phenol-H ²	190-210	Intramolecular exchange with <i>o</i> - and <i>p</i> -positions. Kinetic study.	9
C_6H_6O	Phenol	phenol-H ²	200	50 hrs.	Intramolecular exchange with <i>o</i> - and <i>p</i> -positions complete in 50 hrs.	10, 16
C_6H_6O	Phenol	H ₂ O	Repeated treatment gave phenol-H ² .	18
$C_6H_6O_2$	Resorcinol	H ₂ O	Repeated recrystallization of resorcinol from water-H ₂ gave $C_6H_3H_2^2(OH^2)_2$ and $C_6H_4(OH^2)_2$.	11
$C_6H_6O_2$	Resorcinol	H ₂ O	12
$C_6H_6O_2$	Resorcinol	H ₂ O	Four recrystallizations from water-H ₂ gave a mixture of $C_6H_2H_2^2(OH^2)_2$, $C_6H_3H_2^2(OH^2)_2$ and $C_6H_4(OH^2)_2$.	13
$C_6H_6O_3$	Phloroglucinol	H ₂ O	Phloroglucinol was dried <i>in vacuo</i> at 110° after recrystallization from water. Exchange number 4.713.	14
$C_6H_6O_3$	Phloroglucinol	H ₂ O	Rapid exchange of all hydrogens.	11
$C_6H_6O_3$	Pyrogallol	H ₂ O	11
C_7H_8O	<i>p</i> -Cresol	$C_2H_5OH^2$	Exchange of 1 H atom.	15
C_7H_8O	<i>p</i> -Cresol	$C_2H_5OH^2$	H ₂ SO ₄	22 hrs.	Exchange of 2.89 H atoms (theory, 3).	11
C_7H_8O	<i>p</i> -Cresol (40 g.)	H ₂ O (25 ml.)	HClO ₄	20	10 hrs.	Two phase process with <i>p</i> -cresol dissolved in 30 ml. of CCl ₄ . Exchange equilibrium was not reached even in <i>o</i> -positions.	20
C_7H_8O	Anisole	phenol-H ²	200	When heated in a sealed tube, there is migration of deuterium into the nuclei of both the phenol and anisole.	16, 10
$C_7H_8O_2$	Orcinol	H ₂ O	Rapid exchange of hydroxyl H atom, slow exchange of nuclear H atoms.	11
$C_8H_{10}O$	Phenetole	phenol-H ²	200	Same procedure and result as with anisole, above.	16, 10
C_8H_9ClO	4-Chloro-3,5-xylene	$C_2H_5OH^2$	H ₂ SO ₄	60	342 hrs.	Exchange number, 2.56.	15

(Continued)

TABLE XVI, 28 (Continued)

Formula	Compound	Isotope source	Catalyst	Temp., °C.	Time	Notes	Ref.
C ₈ H ₁₀ O	3,4-Xylenol	C ₂ H ₅ OH ²	H ₂ SO ₄	60	120 hrs.	Exchange number, 2.71.	15
C ₉ H ₁₀ O	5-Indanol	C ₂ H ₅ OH ²	H ₂ SO ₄	60	90 hrs.	Exchange number, 2.63.	15
C ₁₀ H ₈ O	2-Naphthol	C ₂ H ₅ OH ²	H ₂ SO ₄	110	51.5 hrs.	Exchange number, 1.97 (theory, 2).	15
C ₁₀ H ₈ O	1-Naphthol	C ₂ H ₅ OH ²	H ₂ SO ₄	110	3.5 hrs.	Exchange number, 2.93 (theory, 3).	15
C ₁₀ H ₁₂ O	5,6,7,8-Tetrahydro-2-naphthol	C ₂ H ₅ OH ²	Exchange number, 1.10.	15
C ₁₀ H ₁₂ O	5,6,7,8-Tetrahydro-2-naphthol	C ₂ H ₅ OH ²	H ₂ SO ₄	60	180 hrs.	Exchange number, 2.68.	15
C ₁₄ H ₁₀ O ₂	Alizarin	H ₂ O	Exchange of 2 H atoms per molecule.	17

References to Table XVI, 28, DEUTERIUM EXCHANGE WITH PHENOLS

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TABLE XVI, 29
Deuterium Exchange with Miscellaneous Compounds

Formula	Compound	Amount	Isotope source	Amount	Catalyst	Temp., °C.	Time	Notes	Ref.
$\text{CH}_3\text{O}_3\text{SNa}$	Sodium methane-sulfonate	...	H_2O	...	alkali	100-183	...	Rate expression is $\log k_1 (\text{H}^2) = 12.00 - (5630/T)$.	1
$\text{C}_2\text{H}_3\text{N}$	Acetonitrile	...	H_2O	...	alkali	room	12
$\text{C}_2\text{H}_3\text{O}_2\text{K}$	Potassium acetate	...	CH_3COOH^2	150	28-30 hrs.	Equilibrium with H atoms of methyl group.	13
$\text{C}_2\text{H}_6\text{SO}_2$	Methylsulfone	...	H_2O	...	1 N NaOH ²	0-63.5	...	Rate expression is $\log k_1 (\text{H}^2) = 13.23 - (4035/T)$.	1
$\text{C}_3\text{H}_3\text{N}_3\text{O}_3$	Cyanuric acid	...	H_2O	room	24-48 hrs.	...	10
$\text{C}_4\text{H}_4\text{N}_2\text{O}_3$	Barbituric acid	0.4282 g.	99.6% H_2O	2.3510 g.	...	100	20 min.	Product contained 3,365 H^2 atoms per molecule.	2
$\text{C}_4\text{H}_6\text{O}_2\text{Zn}$	Zinc acetate	...	CH_3COOH^2	150	...	Product is zinc acetate- H_2^2 ; half period of exchange is 60 min.	11
$\text{C}_4\text{H}_6\text{O}_3$	Acetic anhydride	...	CH_3COOH^2	150	24 hrs.	Equilibrium with H atoms of methyl group.	13
$\text{C}_4\text{H}_6\text{O}_4\text{Pb}$	Lead acetate	...	CH_3COOH^2	150	200 hrs.	Equilibrium with H atoms of methyl group.	13
$\text{C}_4\text{H}_8\text{N}_2\text{O}_2$	Dimethylglyoxime	...	H_2O	Compound was recrystallized from water- H_2^2 ; the oxime hydrogens exchanged.	3
$\text{C}_4\text{H}_8\text{N}_2\text{O}_2$	Dimethylglyoxime	...	H_2O	Exchanges 8 H atoms per molecule.	4
$\text{C}_4\text{H}_{10}\text{O}_3$	<i>t</i> -Butyl peroxide	...	H_2O	1 wk.	The purified peroxide contained 63 ± 1 mole per cent of OH^2 bonds.	5

$C_4H_{12}O_8Si$	Silicoacetic anhydride	CH_3COOH^2	100	14-25 hrs.	Exchange of H atoms in methyl group.	14
$C_5H_5N_5$	Adenine	500 mg.	H_2O	25 ml.	reduced PtO_2	100	18 hrs.	Product contained 0.91 ± 0.2 atoms of H^2 per molecule.	6
$C_6H_{10}N_2O_7$	1,2-Cyclohexanedione dioxime	H_2O	Compound was recrystallized from water- H_2^2 ; the oxime hydrogens exchanged.	3
$C_7H_{12}N_2O_2$	1,2-Cycloheptanedione dioxime	H_2O	Compound was recrystallized from water- H_2^2 ; the oxime hydrogens exchanged.	3
C_8H_6N	Cyclohexylideneacetone-trile	1 g.	$C_2H_5OH^2$	2.5 ml.	0.1 N ethoxide	25	0.02 hr.	Product contained 0.174 atom % H^2 .	7
C_8H_6N	1-Cyclohexenyl-acetonitrile	1 g.	$C_2H_5OH^2$	2.5 ml.	0.1 N ethoxide	25	3.0 hrs.	Product contained 3.748 atom % H^2 .	7
$C_{10}H_{13}NO_2$	Phenacetin	0.1086 g.	50% H_2O	9.5845 g.	Product contained 0.445 H^2 atoms per molecule.	2
$C_{14}H_{14}N_3O_3Na$	Methyl orange	1 g.	H_2O	1 ml.	0.01 ml. 36 N H_2SO_4	100	11 days	Extensive decomp.; partition ratio = 1.00, assuming 4 exchangeable H atoms.	8
$C_{16}H_{18}ClN_3S$	Methylene blue	1 g.	H_2O	1 ml.	0.01 ml. 36 N H_2SO_4	100	7 days	Partition ratio = 1.22, assuming 4 exchangeable H atoms.	8
$C_{21}H_{22}N_2O_2 \cdot HNO_3$	Strychnine nitrate	H_2O	After recrystallization from water- H_2^2 , product contained 0.8257 atoms H^2 per molecule.	9

(Continued)

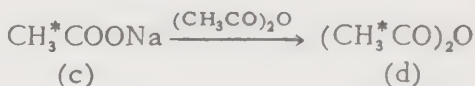
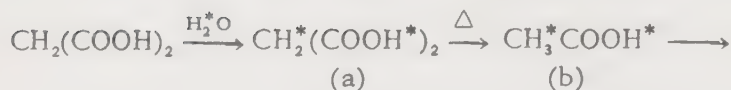
TABLE XVI, 29 (Continued)

Formula	Compound	Amount	Isotope source	Amount	Catalyst	Temp., °C.	Time	Notes	Ref.
$C_{12}H_{14}N_2O_4 \cdot HNO_3$	Vomicin nitrate	H ₂ O	After recrystallization from water-H ₂ , the product contained 2.8457 atoms H ² per molecule.	9
$C_{25}H_{30}N_3Cl$	Crystal violet	1 g.	H ₂ O	1 ml.	0.01 ml. 36N H ₂ SO ₄	100	7 days	Partition ratio = 1.22, assuming 6 exchangeable H atoms.	8
$C_{32}H_{22}N_6O_6S_2Na_2$	Congo red	1 g.	H ₂ O	1 ml.	0.01 ml. 36N H ₂ SO ₄	100	11 days	Partition ratio = 0.38, assuming 8 exchangeable H atoms.	8

References to Table XVI, 29, DEUTERIUM EXCHANGE WITH
MISCELLANEOUS COMPOUNDS

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- ⁴L. V. Korchagin and M. I. Urizko, *J. Phys. Chem. (U.S.S.R.)* 14, 1566 (1940); Chem. Abstracts, 36, 5413 (1942).
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- ¹⁰R. Newman and R. M. Badger, *J. Am. Chem. Soc.*, 74, 3545 (1952).
- ¹¹N. Yamada, K. Suma and T. Takeuchi, *J. Chem. Soc. Japan, Pure Chem. Sect.*, 74, 1018 (1953); through Chem. Abstracts 48, 9794 (1954).
- ¹²L. F. Thomas, E. I. Sherrard and J. Sheridan, *Trans. Faraday Soc.*, 51, 619 (1955).
- ¹³G. P. Miklukhin and A. F. Rekasheva, *Doklady Akad. Nauk. S.S.S.R.*, 101, 881 (1955); through Chem. Abstracts, 49, 12090 (1955).
- ¹⁴I. G. Khaskin, *Sbornik Statei Obshechi Khim.* 2, 1530 (1953); through Chem. Abstracts, 49, 5425 (1955).

B. TRITIUM COMPOUNDS

BIS(ACETIC- H_3^3) ANHYDRIDE

P. Avivi, S. A. Simpson, J. F. Tait and J. K. Whitehead, *Proceedings Radio-isotope Conference*, Vol. I, Academic Press Inc., Publishers, New York, 1954, p. 313.

A. Procedure

(a) *Malonic- H_2^3 Acid- H_3^3* . Into a reaction vessel containing 416 mg. (4 mmoles) of malonic acid is distilled *in vacuo* 217 mg. (12 mmoles) of water- H_2^3 . The reaction vessel is a 60-ml. tube equipped with a stopcock and a ground joint. The mixture is heated at 80° in a tubular oven for 8 hours (Note 1).

(b) *Acetic- H_3^3 Acid- H^3* . Following the heating period, a plug of glass wool is placed in the neck of the reaction vessel, which is then connected to a vacuum manifold. The malonic- H_2^3 acid- H_2^3 is decomposed by heating the tube with a gas burner. The carbon dioxide and acetic- H_3^3 acid- H^3 produced are collected in U-tube traps cooled in liquid nitrogen. The liquid nitrogen baths are replaced with Dry Ice-alcohol baths, and the carbon dioxide is distilled off under vacuum.

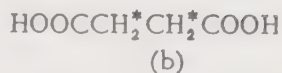
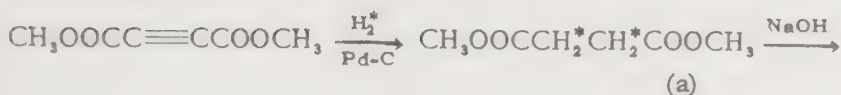
(c) *Sodium Acetate- H_3^3* . The acetic- H_3^3 acid- H^3 is then distilled back into the reaction tube and neutralized with 2 *N* sodium hydroxide and phenolphthalein indicator. The sodium acetate- H_3^3 is made anhydrous by fusion under vacuum.

(d) *Bis(acetic- H_3^3) Anhydride*. To the anhydrous sodium acetate- H_3^3 is added 2 mmoles of acetic anhydride, and the mixture is heated in a sealed tube at 140° for 4 hours. Under these conditions the exchange goes to completion, and the isotopic yield is 53% (Note 2).

B. Notes

1. When the water- H_2^3 was distilled off and assayed, it was found that all the hydrogen atoms of malonic acid had participated in the exchange.

2. The specific activity of the product was 7.9 mc. per mole, which was 53% of the theoretical value if all the hydrogen atoms of the malonic acid exchanged.

SUCCINIC- H_3^3 ACID

D. L. Williams and A. R. Ronzio, U.S. Atomic Energy Comm. Report, AECU 2126; Nuclear Sci. Abstr., 6, 5075 (1952).

A. Procedure

(a) *Methyl Succinate- H_3^3* . The tritiation of methyl acetylenedicarboxylate is done in an all-glass apparatus, Figure XVI, 11. Wherever possible, stopcocks are eliminated and direct seals are made. With the tube provided for attaching the reaction flask sealed off at a, the charcoal

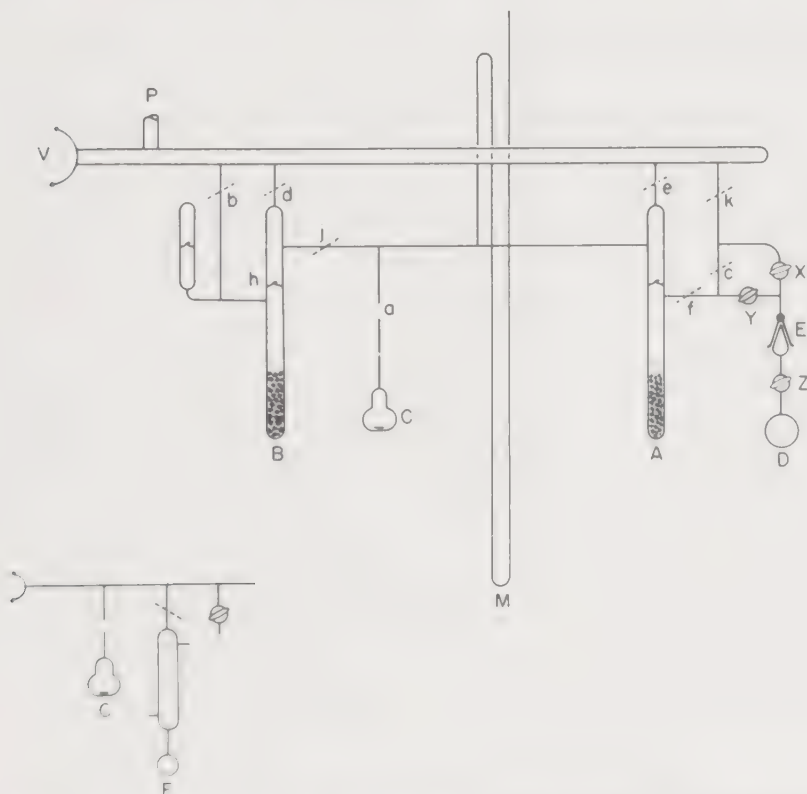


Fig. XVI, 11 Apparatus for the hydrogenation of methyl acetylenedicarboxylate with pure tritium (D. L. Williams and A. R. Ronzio). A and B, charcoal filled traps; C, reaction flask; D, 100-ml. flask containing tritium; E, standard-taper joint; M, manometer; P, to Pirani gauge; V, to vacuum; F, hydrolysis flask with sealed-on-condenser.

(Note 1) in the traps A and B is degassed under vacuum at about 300° for 24 hours. When the pressure in the system is down to 1.5 microns, seals are made at b and c. The reaction flask C, containing a glass-enclosed magnetic stirring bar, 25.9 mg. of catalyst (Note 2) and 145 mg. (1.02 mmoles) of methyl acetylenedicarboxylate, is cooled in liquid nitrogen and sealed to the system at a. With C still in the liquid nitrogen bath, the inner system is evacuated to 1.5 microns, and seals are made at d and e. The 100-ml. flask D, containing 60.8 ml. (S.T.P.) of hydrogen- H_2^3 (Note 3), is attached at E with Apiezon W cement. The section at joint E is evacuated through stopcock X. Then with X closed, Y open, and Z open, the hydrogen- H_2^3 is transferred to charcoal trap A immersed in liquid nitrogen. A seal is made at f, and breakoff seal g is broken to allow hydrogen- H_2^3 to enter the reaction flask. With C still immersed in liquid nitrogen, A is warmed to room temperature. Then with flask C also at room temperature, the initial pressure reading is taken (Note 4).

The reaction mixture is stirred continuously for 5 days, at which time the rate of hydrogen- H_2^3 uptake has dropped practically to zero (Note 5). With C immersed in liquid nitrogen, breakoff seal h is broken, and the excess hydrogen- H_2^3 is adsorbed on the charcoal in trap B, also immersed in liquid nitrogen. When the pressure in the system is approximately zero, as registered by the manometer, a seal is made at j. Flask C is sealed off at a and resealed to the small manifold shown in Figure III, 11 for isolation of the product. The methyl succinate- H_4^3 is vacuum-distilled for 5 days into hydrolysis flask F cooled with liquid nitrogen (Note 6). When no more product is collected, the hydrolysis flask F, with condenser attached, is removed from the manifold at k (Note 7).

(b) *Succinic- H_4^3 Acid*. The methyl succinate- H_4^3 is hydrolyzed during 1 hour with 3 ml. of 10% sodium hydroxide at reflux temperature. The basic solution is then transferred to an ether extractor, acidified with 6 N hydrochloric acid and extracted with ether for 48 hours. The ether is removed from the crude succinic acid by distillation; the receiver is cooled to -78° and is connected to a trap immersed in liquid nitrogen (Note 8). The crude acid is dissolved in dry acetone and filtered through a thin bed of activated carbon in a fritted glass funnel. Evaporation of the solvent under partial vacuum leaves 18.4 mg. (14.3%) of succinic- H_4^3 acid (Notes 9 and 10).

B. Notes

1. A special cocoanut shell charcoal, about 40 mesh, was used.
2. The catalyst of 5% palladium-on-carbon was reduced with formaldehyde,¹ rather than hydrogen, so that the hydrogen- H_2^3 was not diluted by adsorbed hydrogen.

3. Purity of the hydrogen- H_2^3 was 99.6%; therefore, the total activity was 158 curies.

4. From the known volume of hydrogen- H_2^3 introduced and the pressure and temperature, the volume of reaction apparatus was calculated. Following the change in pressure then permitted calculation of the hydrogen- H_2^3 consumed in the reaction.

5. After a few minutes the rate of hydrogen- H_2^3 consumption was much slower than in similar experiments with hydrogen. After 16 hours the formation of a nonvolatile glass-like "polymer" was apparent. The polymerization of methyl acetylenedicarboxylate by the relatively large amount of beta radiation present is to be expected in view of radiation effects² on acetylene.

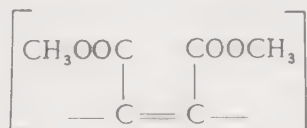
6. Apparently volatilization of the ester was retarded by the viscous "polymer" since this isolation normally took about 24 hours.

7. The ester was not weighed, but the yield was practically equal to that of succinic acid, in the preliminary experiments.

8. There was activity in the methyl alcohol obtained in the distillate.

9. The product, a white crystalline solid, appeared gummy in 15 days and had changed to a glass-like material after about 30 days.

10. The amount of hydrogen- H_2^3 required for the formation of 0.146 mmole of succinic acid is 6.5 ml. (S.T.P.). The total hydrogen- H_2^3 consumption was 25.4 ml. (S.T.P.); therefore, 18.9 ml. (S.T.P.) of hydrogen- H_2^3 was consumed in saturating the polymer. If the polymer unit is assumed to be

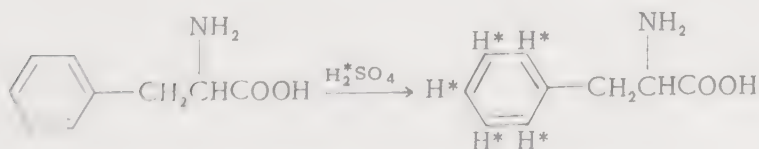


the calculated amount of hydrogen- H_2^3 required for its complete saturation is 19.65 ml. (S.T.P.).

¹A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., New York, 1948, p. 990.

²S. C. Lind, *The Chemical Effects of Alpha Particles and Electrons*, The Chemical Catalogue Co., New York, 1928, p. 158.

PHENYL- H_3^3 -ALANINE (2-Amino-3-phenyl- H_3^3 -propionic Acid)



S. Gurin and A. M. Delluva, *J. Biol. Chem.*, 170, 545 (1947).

A. Procedure

Phenylalanine, 20 g., is heated for 6 days at 50° with 8.5 g. of 84% sulfuric acid- H_2^3 (Note 1). The phenyl- H_2^3 -alanine is isolated according to the following procedure of Moss and Schoenheimer,¹ and 1.8 to 1.9 g. of recrystallized product is recovered. At the end of the heating period, the solution is diluted with about six times its volume of water, and the pH adjusted to 5.5 by dropwise addition of 9 M sodium hydroxide solution. During neutralization, vigorous mechanical stirring is employed, and the temperature is maintained between 26 – 29° (Note 2). The phenylalanine precipitates suddenly, when the pH is approaching 5, as white, apparently amorphous, small particles. After standing for one-half hour at 28° , the product is collected on a filter and washed with two portions of ice-cold water. The product is twice crystallized from water after treatment with a very small amount of carbon. The lustrous, colorless plates are dried *in vacuo* over phosphorus pentoxide (Note 4).

B. Notes

1. The sulfuric acid- H_2^3 was made from sulfur trioxide and water- H_2^3 .
2. Thus, the separation of sodium sulfate is prevented.
3. Work with deuterium¹ has demonstrated that all of the hydrogens of the benzene ring are exchanged under these conditions. The deuterium so introduced was stably bound, the isotopic content being completely unchanged by boiling the product for long periods with 6 N hydrochloric acid in ordinary water.
4. The final mother liquors from the crystallization and the sodium sulfate-containing mother liquor from the isoelectric precipitation were combined, heated to about 60° and treated with copper acetate. The light-blue phenyl- H_2^3 -alanine copper salt was washed with water and dried over phosphorus pentoxide *in vacuo*. In this manner, the total yield of phenylalanine recovered was about 92%.

¹A. R. Moss and R. Schoenheimer, J. Biol. Chem., 135, 421 (1940).

H^3 -OLEIC ACID (H^3 -9-Octadecenoic Acid)

D. Kritchevsky, R. F. J. McCandless, J. E. Knoll and M. L. Eidinoff, J. Am. Chem. Soc., 77, 6655 (1955).

A. Procedure

(a) *Glyceryl Tris(H^3 -oleate)*, (H^3 -Triolein). A suspension of 1 g. of platinum oxide catalyst in 1 ml. of water- H_2^3 , contained in a glass tube, is reduced in a stream of hydrogen gas. After the tube is thoroughly

flushed with nitrogen, 6 g. of glyceryl oleate, 15 mg. of potassium hydroxide and an additional 5 ml. of water- H_2^3 are added. The tube is evacuated, sealed and then heated at 130° for 28 hours, with constant shaking. Following the heating period, the organic layer is dissolved in ether, and the acidic material is separated from the neutral triolein on an IRA-400 ion exchange column¹ (Note 1). The eluted triolein, 5.2 g., has an iodine number of 85 and d_4^{20} 0.915 (Note 2).

(b) H^3 -Oleic Acid, (H^3 -9-Octadecenoic Acid). The above H^3 -triolein is heated under reflux for 5 hours with concentrated alcoholic potassium hydroxide, in an atmosphere of nitrogen. The reaction mixture is acidified and extracted with ether (Note 3). The ether solution is passed through a column of IRA-400 resin, and the adsorbed H^3 -oleic acid is eluted with alcoholic hydrogen chloride (Note 4).

B. Notes

1. The specific activity of the eluted triolein, measured by gas counting,² was 16.9 μ c. per g.

2. The iodine number of the starting material was 85.3, and the literature lists d 0.915 (15°)³ and 0.9152 (20°).⁴

3. The glycerol, obtained by distillation of the basic aqueous phase to dryness under vacuum and extraction of the residue with hot acetone, had an activity of 9 μ c. per mole.

4. The activity of the H^3 -oleic acid was 14.8 μ c. per g. To ensure that none of the activity was due to hydrogenation of double bonds, the acid was subjected to paper chromatography using the method of Savary,⁵ which gives wide separation of oleic and stearic acids. Descending chromatography on single strips of Whatman No. 1 paper was used, with methanol-water (8:2) as the developing solvent. After development of the chromatogram, no color with Rhodamine B spray and no activity were found at the origin. The H^3 -oleic acid gave R_f values of 0.58–0.93.

¹J. Cason, G. Sumrell and R. S. Mitchell, J. Org. Chem., 15, 850 (1950).

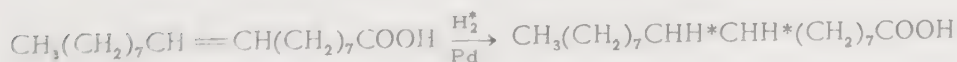
²M. L. Eidinoff, J. E. Knoll, D. K. Fukushima and T. F. Gallagher, J. Am. Chem. Soc., 74, 5280 (1952).

³H. Pottevin, Compt. rend., 138, 378 (1904).

⁴S. T. J. Tromp, Rec. trav. chim., 41, 278 (1922).

⁵P. Savary, Bull. soc. chim. biol., 36, 927 (1954).

OCTADECANOIC-9,10- H_2^3 ACID (Stearic-9,10- H_2^3 Acid)



A. Procedure

Stearic-9,10- H_2^3 acid is prepared on a micro-scale using the vacuum apparatus shown in Figure XVI, 12. Elaidic acid, 14.4 mg., and 3.9 mg. of palladium-black, are introduced into the reaction vessel, V, which is then sealed to the apparatus. The system is cautiously evacuated (Note 1), and 0.05 ml. of dry dioxane is added by distillation *in vacuo*. Then 1.15 ml. of hydrogen- H_2^3 , from the bulb Tr, is measured out in the calibrated section of the Toepler pump, C_T , and compressed into the reaction vessel, V. This is done with the reaction vessel still cooled in liquid air; the mercury in R is lowered to the bottom of the tube, and the hydrogen in the pump is forced past stopcock S_{58} . The mercury in R is then raised to the top of the bulb, thus compressing the gas to about 1 atmosphere (Note 2). After 46 hours at room temperature, the reaction is 96% complete (Note 3). The reaction vessel is removed from the vacuum system, and the mixture is extracted with ether. Catalyst is removed by

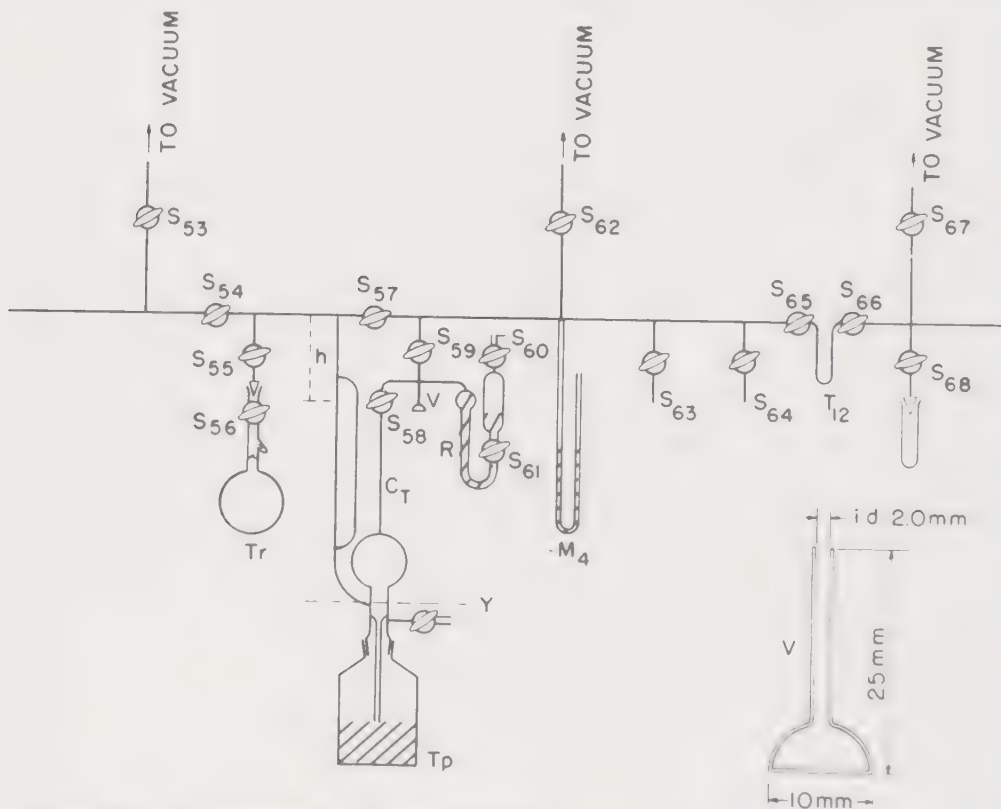


Fig. XVI, 12 Apparatus for hydrogenation of unsaturated bonds on a micro-scale (R. F. Glascock). C_T , calibrated tube on Toepler pump; h , maximum head of mercury attainable for compression of gas past S_{58} ; M_4 , manometer; S_{54} - S_{68} , stopcocks; T_{12} , U-trap; Tr, bulb of tritium; T_p , Toepler pump; R, mercury reservoir (for altering capacity of adjoining apparatus); V, reaction vessel (details enlarged); Y, see Note 2.

centrifugation, and the supernatant liquid is evaporated to dryness. The yield of stearic-9,10- H_2^3 acid is 14.0 mg. (97%).

B. Notes

1. The catalyst may be carried into other parts of the apparatus as gas is desorbed.

2. Since the volume of the reaction vessel is so small (about 1 ml.) compared to the volume of the Toepler pump bulb (250 ml.), the volume of gas taken may be checked by setting the level of mercury in the pump just above the junction of the bulb with the side tube at Y and opening stopcock S_{58} . The stopcock is immediately closed, the mercury level in the pump is adjusted, and a reading is taken. No dead-space correction factor need be applied, and close agreement with the former reading should be observed. The gas is returned to V, as above, and the liquid air is removed. The progress of the reaction may be followed by measuring the residual hydrogen at intervals, in this manner.

3. The reaction proceeds much more slowly in this apparatus than in one in which stirring or shaking of the mixture is possible and in which the gas pressure is not allowed to diminish.

¹I. M. Heilbron, W. A. Sexton and F. S. Spring, J. Chem. Soc., 1929, 926.

H^3 -STEARIC ACID

D. J. Rosenthal and D. Kritchevsky, U.S. Atomic Energy Comm. Report, UCRL-1331, Nuclear Sci. Abstr., 5, 5156 (1951).

A. Procedure

The general method of Van Heyningen, Rittenberg and Schoenheimer,¹ for deuterium labeling of fatty acids, is used (Note 1).

In a glass tube, 1 g. of platinum oxide catalyst² suspended in 2 ml. of dilute water- H_2^3 (Note 2) is reduced in a stream of hydrogen. The hydrogen is flushed from the tube with nitrogen (several replacements), and 7.5 g. of stearic acid, 0.100 g. of potassium hydroxide and 9 ml. of additional water- H_2^3 are added. With the contents frozen, the tube is sealed and then shaken at 135° for 200 hours. After the reaction period, the tube is opened and attached to a vacuum system, and the liquid is distilled off *in vacuo* (Note 3). The dry residue is dissolved in ether-benzene, acidified, and filtered through Celite to remove the catalyst. Evaporation of the solvent leaves 7.5 g. of crude stearic acid, which is recrystallized from ethanol-water to obtain 7.1 g. (95%) of pure product (Note 4).

B. Notes

1. By this method, it is possible to obtain 36% of the equilibrium amount of tritium incorporated into the fatty acid.

2. Use of water- H_2^3 at this stage results in an unnecessary loss of tritium.

3. Of the initial water charge, 10.9 ml. (99%) is recovered.

4. According to the work with deuterium,¹ stably bound tritium is introduced at many and perhaps all of the carbons.

C. Other Preparations

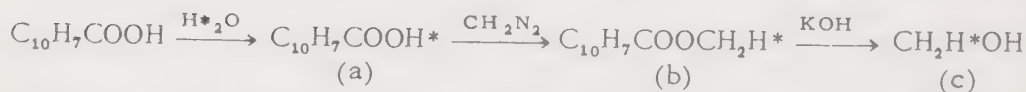
H^3 -Stearic acid has been prepared³ by the hydrogenation of linoleic acid, dissolved in ethyl acetate containing water- H_2^3 , with ordinary hydrogen and a platinum catalyst.

H^3 -Palmitic acid has also been prepared³ by exchange. The acid, dissolved in dilute potassium hydroxide solution containing water- H_2^3 and 1.0 g. of platinum oxide catalyst, was heated at 135° for 6 days.

¹W. E. Van Heyningen, D. Rittenberg and R. Schoenheimer, J. Biol. Chem., 125, 495 (1938).

²Organic Syntheses, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

³R. G. Gould, unpublished work.

METHANOL-1- H_1^3 

W. G. Verly, J. R. Rachele, V. du Vigneaud, M. L. Eidinoff and J. E. Knoll, J. Am. Chem. Soc., 74, 5941 (1952).

A. Procedure

(a) *2-Naphthoic Acid- H^3* . 2-Naphthoic acid is dissolved in peroxide-free, dry dioxane and water- H_2^3 is added to this solution, with thorough mixing. The carboxyl-labeled acid is then isolated by removing the solvent *in vacuo*.

(b) *Methyl- H_1^3 2-Naphthoate*. The 2-naphthoic acid- H^3 , dissolved in dry ether, is added to an ethereal solution of diazomethane¹ in excess. After the excess of diazomethane and the solvent are removed by evaporation, the methyl- H_1^3 2-naphthoate is transferred to a glass tube.

(c) *Methanol-1- H_1^3* . A slight excess of powdered, dry potassium hydroxide is mixed with the ester, and some carrier methanol is added. The tube is cooled, sealed, and heated in a boiling water-bath for several hours. The tube is then opened, and the methanol-1- H_1^3 is distilled, *in vacuo* into a cooled trap.

B. Other Preparations

By a procedure similar to that described, Melander² prepared methyl- H_1^3 benzoate and methanol- 1-H_1^3 . Isotopic dilution occurred, since a mixture of ordinary benzoic acid and water- H_2^3 was treated with diazomethane.

In their synthesis of methyl- H_2^3 iodide, Harman, *et al.*,³ prepared, but did not isolate, what was probably a mixture of methanol- $\text{H}^3\text{-}1\text{-H}_2^3$ and methanol- H^3 , by the tritiation of methyl formate over a copper chromite catalyst.

¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 165.

²L. Melander, *Arkiv Kemi*, 3, 525 (1951).

³D. Harman, T. D. Stewart and S. Ruben, *J. Am. Chem. Soc.*, 64, 2293 (1942).

ETHANOL- 1-H_2^3

METHOD I

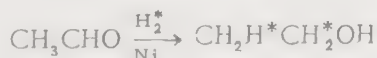


L. Kaplan, *J. Am. Chem. Soc.*, 76, 4645 (1954); K. E. Wilzbach and L. Kaplan, *ibid.*, 72, 5795 (1950).

A. Procedure

Into an evacuated flask at -195° and containing 0.302 g. (7.9 mmoles) of lithium aluminum hydride- H_4^3 is distilled 15 ml. of dry ether and 1.845 g. (21.0 mmoles) of ethyl acetate. The flask is warmed in a bath at -22° and, after the reaction subsides, the mixture is kept at room temperature for 2 hours with occasional shaking. The ether and excess ethyl acetate (Note 1) are removed *in vacuo*, and 2 g. of water is distilled onto the residue. After the reaction mixture stands for 2 hours at room temperature, the volatile material is distilled *in vacuo* successively onto two 15-g. portions of Drierite, and is then fractionated through a series of traps on a vacuum line. The yield of product is 1.138 g. (78%, based on LiAlH_4^3) (Notes 2 and 3).

METHOD II



L. Melander, *Arkiv Kemi*, 3, 525 (1951).

A. Procedure

Freshly distilled acetaldehyde is hydrogenated at atmospheric pressure and about 170° with hydrogen- H_2^3 over a catalyst of nickel supported on

kieselguhr.¹ By proper adjustment of the rate of flow over the catalyst and the ratio of hydrogen to acetaldehyde, it is possible to get consumption of about three-fourths of the hydrogen and introduction of about 60% of the tritium into the resulting ethanol.

The ethanol is distilled in a small column of the Podbielniak type, and the fraction of boiling range 77-77.9° is collected. To remove labile tritium present in the hydroxyl group, the ethanol fraction is twice distilled from 10 volumes of ethylene glycol (Note 4). The ethanol fraction (material boiling up to 194°) is then fractionated in a Podbielniak column, and product of boiling range 77.4-78.6° is collected (Note 5).

B. Notes

1. The unreacted ethyl acetate contained a considerable amount of tritium.

2. The product had vapor pressures of 12.5 mm. at 0° and 43.5 mm. at 20°.

3. The ethanol-1-H³₂ was used in a study of hydrogen isotope effect in the bromine oxidation of ethanol. At 37.5° the rate of oxidation of ethanol-1-H³₂ was 0.57 times that of ethanol; this corresponds to a ratio of about 0.15 for the rates of removal of tritium and hydrogen atoms from the methylene carbon.

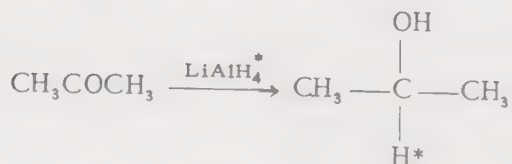
4. It was estimated from the tritium present in the second glycol portion that less than 0.3% of the tritium in the final ethanol was in the hydroxyl group.

5. Oxidation of a portion of the ethanol indicated that 11.3% of the tritium was present in the methyl group. A 1.5-ml. aliquot of the product was oxidized to H³-acetic acid with sodium dichromate and dilute sulfuric acid. From the solution, made just neutral with sodium hydroxide, sodium uranyl H³-acetate was crystallized upon the addition of uranyl nitrate.

¹L. W. Covert, R. Connor and H. Adkins, J. Am. Chem. Soc., 54, 1651 (1932).

²*Organic Reactions*, Vol. VI, Wiley, New York, 1951, p. 469.

2-PROPANOL-2-H³



L. Kaplan, J. Am. Chem. Soc., 77, 5469 (1955).

Procedure

Into a 50-ml., long-necked flask, containing a small glass-enclosed magnet, is introduced 0.273 g. (7.2 mmoles) of lithium aluminum hydride-

H_4^3 . The flask is attached to a vacuum apparatus, 20 ml. of dry ether is introduced, by distillation under vacuum, and the mixture is stirred for 1 hour. After the flask is cooled with liquid nitrogen and evacuated, 3.0 ml. of dry acetone is introduced. The mixture is kept at -20° for one-half hour and is then stirred at room temperature for 1 hour. The ether and excess acetone are removed *in vacuo*, and 1.1 g. of water is distilled onto the residue. After the mixture is kept for 2 hours at room temperature, the volatile material is distilled into a flask containing 15 g. of Drierite. After sufficient time is allowed for drying, the flask is placed in a bath at 0° and the product is distilled *in vacuo*. The yield of 2-propanol-2- H^3 , with a vapor pressure of 10 mm. at 0° , is 1.38 g. (80% based on $LiAlH_4$; 77% based on tritium activity).

AROMATIC H^3 -AMINES

B. J. Fontana, J. Am. Chem. Soc., 64, 2503 (1942).

Procedure

In general, 1 g. of the compound is mixed with 1 ml. of water- H_2^3 and 0.01 ml. of 36 *N* sulfuric acid, unless otherwise noted. The mixture sealed in a tube is heated at 100° , usually for 7 days. The water is then distilled off *in vacuo* and purified by distillation *in vacuo* from potassium permanganate and sodium carbonate. The amount of exchange is determined by the change in density¹ of the water. The exchange data with several aromatic amines are given in Table XVI, 30.

TABLE XVI, 30
Tritium Exchange with Aromatic Amines

Compound	<i>n</i>	<i>nk</i>	<i>k</i>
Crystal violet	6	9.18	1.53 ^a
Methylene blue	4	5.21	1.30 ^a
Benzidine	8	2.86	0.33
Benzidine	8	0.90	0.11 ^{b,a}
Benzidine · 1HCl	8	0.60	0.08 ^{c,d}
Benzidine · 2HCl	8	2.90	0.36 ^d

a. Usual heating period plus 6 weeks at room temperature.

b. 0.02 ml. of sulfuric acid catalyst.

c. Solvent is 50% ethanol and 50% water; 10 days at 100° and 3 months at room temperature.

d. No acid catalyst added.

n. The number of exchangeable hydrogens, i.e., the number of unoccupied nuclear positions ortho and para to the amino groups.

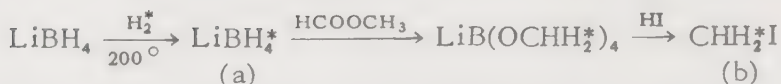
k. Partition ratio of tritium between solute and solvent.

nk. The exchange number.

¹B. J. Fontana and M. Calvin, Ind. Eng. Chem., Anal. Ed., 14, 185 (1942).

Iodomethane- H_2^3

METHOD I



N. H. Smith, K. E. Wilzbach and W. G. Brown, J. Am. Chem. Soc., 77, 1033 (1955).

A. Procedure

(a) *Lithium Borohydride- H_4^3* . An ether solution of purified¹ lithium borohydride is filtered into the reaction flask (Note 1) through a long-stemmed, sintered-glass funnel. After complete removal of the solvent, *in vacuo* at 200° , the weight of hydride is 0.113 g. (5.18 mmoles). The reaction flask, mounted in a horizontal position, is attached to a Toepler pump, and 18.28 ml. (0.82 mmole) of hydrogen, containing 644 mc. of tritium, is introduced. The exchange reaction is effected by immersing the bulb of the flask in a bath at 200° (Note 2). After 97 hours, the tritium content of the gas is 71 mc.; therefore, by difference, the tritium content of the lithium borohydride is 573 mc. (89% of that initially present in the gas).

(b) *Iodomethane- H_2^3* (Note 3). The lithium borohydride- H_4^3 is dissolved in about 10 ml. of tetrahydrofuran, which is vacuum-distilled into the reaction vessel from lithium aluminum hydride. The solution is frozen and 0.629 g. (1% excess) of methyl formate is introduced (Note 4). The reaction mixture is gradually warmed and maintained at 0° for 30 minutes and then at 20° for 1 hour. The solvent is transferred *in vacuo* to a similar reaction flask which contains 20 mg. of nonlabeled borohydride (Note 5); complete removal of solvent is effected by warming the first flask to 60° . By means of a dropping funnel, which has a stem long enough to reach into the bulb of the reaction flask, 25 ml. of hydriodic acid is added to the dry residue. Then, with water flowing through the condenser, the solution is heated at 90° for 2 hours while helium (about 30 ml. per minute) is passed, in turn, through the funnel, the solution, a trap cooled in liquid nitrogen, and a pressure outlet. The trap is warmed to -95° (Note 6) and evacuated to remove material more volatile than methyl iodide. The trap is then warmed in a bath at -63° , and the methyl- H_2^3 iodide is distilled into a storage flask; the yield is 2.775 g. (19.5 mmoles) (Note 7).

METHOD II



D. Harman, T. D. Stewart and S. Ruben, J. Am. Chem. Soc., 64, 2293 (1942).

A. Procedure

The apparatus is designed to allow recovery of unused tritium in a storage bulb (Note 8). After the system is filled with hydrogen and evacuated three times, the reaction mixture is prepared in a storage bulb by admitting methyl formate to a pressure of 7.6 cm. of mercury and transferring tritium with the Toepler pump to give a total pressure of 76 cm. This mixture is passed over the catalyst (Note 9) at a rate of about 10 cc. per minute by allowing mercury to pass from a reservoir into the storage bulb. The resulting mixture of methanol- H^3 -1- H_2^3 and methanol- H^3 is passed through a Zeisel apparatus.² In order to maintain atmospheric pressure in the reservoir, mercury is added through an external stopcock. The methyl- H_2^3 iodide is collected in a liquid-air cooled trap, and unused tritium is recycled to a storage bulb.

The methyl- H_2^3 iodide is made in about 2-g. quantities and 75% yield. The crude material of d_4^{20} 1.5268 melts at about -60° . It is redistilled *in vacuo*, and the vapor is passed over phosphorus pentoxide.

B. Notes

1. The reaction vessel used for the exchange reaction, the reduction of methyl formate and the preparation of methyl iodide was a 50-ml. bulb sealed to a micro-condenser which was sealed at its upper end to a ground-glass joint and a side arm equipped with a stopcock and a joint.

2. The extent of hydrogen exchange was determined, at suitable intervals, by cooling the reaction vessel to room temperature, expanding the gas into a small calibrated bulb and then transferring this aliquot into an ionization chamber for determination of tritium.

3. Preparation of tritium labeled methyl iodide by this procedure more efficiently uses a given amount of tritium than the catalytic reduction of methyl formate (see Method II) and results in less isotopic dilution than in the preparation of methanol *via* diazomethane and water- H_2^3 (see methanol-1- H_1^3).

4. A larger excess is avoided, since there is an exchange of methoxyl groups between the reduction product and unreacted ester.

5. Unreacted methyl formate is reduced in this manner, and a product of lower activity is obtained.

6. This bath was a melt prepared by addition of liquid nitrogen to toluene.

7. The total yield of methyl iodide from the two fractions of methyl formate was 20.3 mmoles (97%). The products were both largely solid at -65° ; the melting point of methyl iodide is -64.4° .

8. A diagram of the apparatus used and details of its manipulation are given by Harman, Stewart and Ruben.

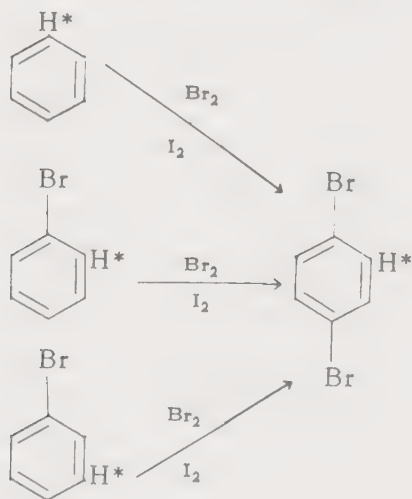
9. The catalyst was prepared by dissolving 120 g. of cupric nitrate in 100 ml. of water and adding a saturated solution containing 60 g. of

potassium carbonate, at a temperature of about 50° . The precipitated basic copper carbonate was filtered off, washed well and dried; 45 g. of the pulverized material was added to 100 cc. of quartz chips (0.5 to 1 mm.) and 50 ml. of chromic acid solution containing 2 g. of CrO_3 . The mixture was well stirred and evaporated to dryness on a steam-bath. The catalyst chamber of about 3.5 cc. capacity was filled with this material, and the catalyst was reduced by passing about 20 cc. of hydrogen per minute over it, at a temperature of 325° , for 6 hours. Tank hydrogen was passed over the catalyst at 160° , the temperature used for the reduction of the ester, for 2 hours prior to each preparation.

¹W. D. Davis, L. S. Mason and G. Stegeman, *J. Am. Chem. Soc.*, 71, 2777 (1949).

²J. B. Niederl and V. Niederl, *Organic Quantitative Micro-Analysis*, John Wiley and Sons, Inc., New York, N. Y., 1938, p. 187.

1,4-DIBROMOBENZENE-2- H^3



L. Melander, *Arkiv Kemi*, 2, 275 (1950).

A. Procedure

The following general procedure is used in the bromination of benzene- H_1^3 and the isomers of H_1^3 -bromobenzene.

In a small flask equipped with a reflux condenser, 5-44 mg. of iodine and 0.7 to 1.0 ml. of bromine (Note 1) are added to 0.50 ml. of benzene- H_1^3 . After some time, when practically all of the mixture has solidified, it is washed successively with sodium hydroxide solution, hot water, dilute sulfuric acid, and again with water. The crude product is recrystallized from alcohol. The final product is collected by filtration and dried *in vacuo* over calcium chloride. Detailed experimental data are

given in Table XVI, 31 (Note 2). Yields are determined by the isotopic dilution method (see 1,3-dinitrobenzene-2,4,5- $H^3_{1/3}$, Note 2).

TABLE XVI, 31
 H^3 -Dibromoaryl Compounds

Starting Compound, 0.5 ml.	Product	M.p. (uncor.)	Catalyst	Br ₂ ml.	Reaction time	Y/Yo **
Benzene- H^3_1	1,4-dibromo-benzene-2- H^3	85-88°	2.3 mg. AlCl ₃	0.62	1 day	0.781
"	"	85-87°	4.5 mg. Fe*	0.70***	"	0.799
"	"	85.5-87.5°	44 mg. I ₂	0.70	"	0.696
1-Bromo-benzene-2- H^3	"	84-86.5°	30.7 mg. AlCl ₃	0.26	"	0.923
"	"	84-86.5°	58.8 mg. I ₂	0.26	2 days	1.015
1-Bromo-benzene-3- H^3	"	84-86.5°	33.8 mg. AlCl ₃	0.26	1 day	0.980
"	"	83-87°	62.5 mg. I ₂	0.26	2 days	0.947
1-Bromo-benzene-4- H^3	H^3 -1,4-dibromobenzene	84-86°	41.2 mg. AlCl ₃	0.30	5 hours	0.238
"	"	81-84°	26.2 mg. AlCl ₃	0.30	3 days	0.474
"	"	85-87°	10.5 mg. Fe*	0.30	45 min.	0.123
"	"	85-87°	36.0 mg. I ₂	0.30	1 day	0.046

*Catalyst was reduced iron powder.

**Y = ratio of isotopic to nonisotopic molecules in product;

Yo = same ratio in starting material.

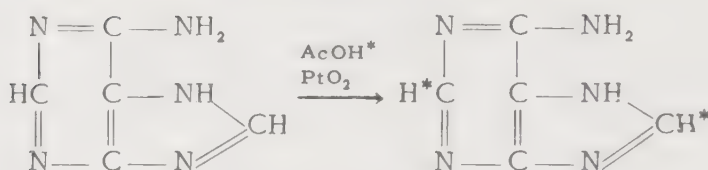
***3.0 ml. CS₂ was added as solvent.

B. Notes

1. With too much bromine and a longer time of reaction, more highly brominated homologues are easily formed.

2. With all three catalysts used in the brominations, hydrogen exchange occurred. This was clearly shown in the bromination of 1-bromo-benzene-4- H^3 , which should give a tritium-free 1,4-dibromobenzene; actually, the tritium in the products ranged from 4.6% to 47.4% of that in the starting material, depending upon the catalyst used and the duration of reaction.

ADENINE-2,8- H^3_2
(6-Aminopurine-2,8- H^3_2)



M. L. Eidinoff and J. E. Knoll, J. Am. Chem. Soc., 75, 1992 (1953).

A. Procedure

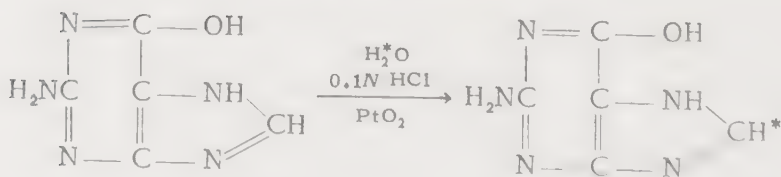
Platinum oxide catalyst, 250 mg., is reduced with ordinary hydrogen in the reaction medium made up of 70% aqueous acetic acid containing acetic acid- H^3 . To the reaction mixture, total volume 25 ml., is added 500 mg. of adenine. After the mixture is frozen, the tube is evacuated and sealed. With shaking, the tube is heated at 100° for 18 hours. The cooled solution is filtered to remove the catalyst, and the filtrate is evaporated to dryness. The product is heated with 1 *N* sodium hydroxide for several minutes and is then precipitated as the hydrochloride by acidification of the hot solution (Note 1). This type of purification replaces tritium, bonded to nitrogen, with ordinary hydrogen; therefore, the tritium remaining in the compound is quite probably bonded to carbon in positions 2 and 8 (Note 2).

B. Notes

1. In another experiment, the exchange medium was water- H^3_2 . The product was purified by recrystallization of the free base from ordinary water. The tritium content of this H^3 -adenine was unchanged after standing in 0.01 *N* hydrochloric acid solution for 2 days at room temperature and after boiling in 1 *N* sodium hydroxide solution for 5 minutes.

2. In a similar experiment with no catalyst present in an acetic acid- H^3 medium, there was no stably bound tritium in the product.

GUANINE-8- H^3
(2-Amino-6-hydroxypurine-8- H^3)



M. L. Eidinoff and J. E. Knoll, J. Am. Chem. Soc., 75, 1992 (1953).

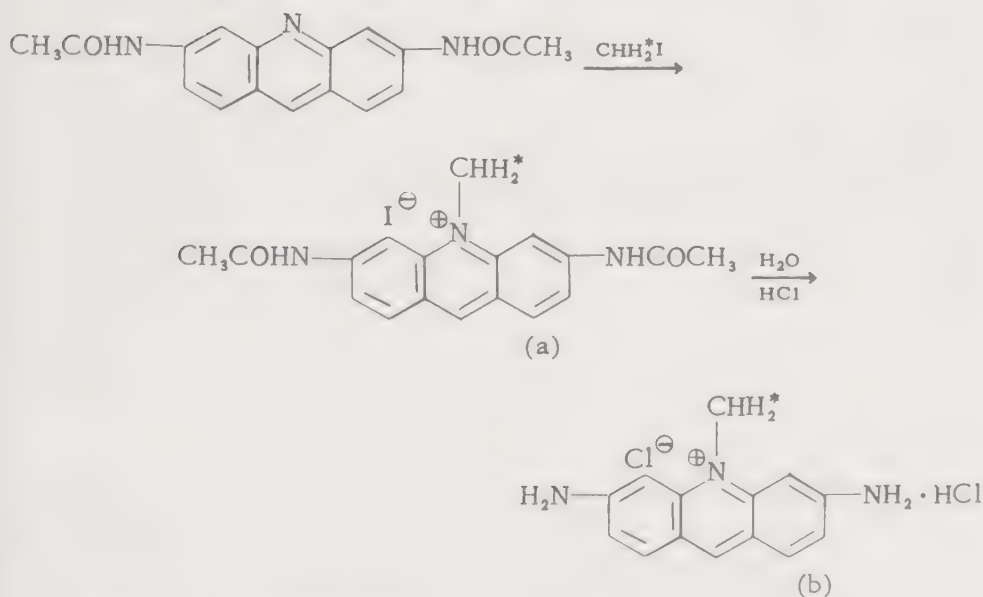
A. Procedure

To a mixture of water- H_2^3 and 0.1 *N* hydrochloric acid is added 500 mg. of guanine and 250 mg. of platinum oxide catalyst. The catalyst is reduced with ordinary hydrogen at room temperature; then the reaction mixture is frozen, and the reaction tube is evacuated and sealed. With shaking, the tube is heated at 100° for 18 hours. Following the heating period, the product is dissolved in hot 1 *N* sodium hydroxide solution and precipitated as the sulfate (Note 1). Exchange of tritium for hydrogen, in non-labile positions, takes place to the extent of 0.6 of the equilibrium value, under these experimental conditions.

B. Notes

1. Since tritium attached to nitrogen or oxygen is readily exchanged, this treatment should leave only position 8 as the site of the isotopic label.

**3,6-DIAMINO-10-METHYL- H_2^3 -ACRIDINIUM
CHLORIDE HYDROCHLORIDE**
(H_2^3 -Acriflavine Hydrochloride)



N. H. Smith, K. E. Wilzbach and W. G. Brown, *J. Am. Chem. Soc.*, 77, 1033 (1955).

A. Procedure

(a) *3,6-Diacetamido-10-methyl- H_2^3 -acridinium iodide*. Into the larger, open arm of an H-shaped reaction vessel (Note 1) is weighed 4.526 g.

(15.43 mmoles) of 3,6-diacetamidoacridine (Note 2). The vessel is then attached to a vacuum system, and 2.775 g. (19.53 mmoles) of methyl- H_2^3 iodide is transferred into the other arm *in vacuo*, with liquid nitrogen (Note 3). While the iodide is still frozen, the reaction vessel is sealed off below the tapered joint. The iodide is then thawed, and the vessel is heated at 50° for 16 hours. At the end of this period, the methylation is about 90% complete (Note 4); the tube is heated at 110° for an additional 48 hours. The excess methyl- H_2^3 iodide is condensed in the smaller arm with liquid nitrogen, the stem above the ground glass joint is snapped off, and the vessel is attached to a vacuum line. Complete removal of volatile material is ensured by heating the larger arm of the vessel to 100° while the latter is evacuated. The weight of the solid product is 6.682 g.; the increase in weight is equivalent to 98.4% methylation of the starting material. From the volatile material is obtained, after it is purified by distillation, 0.616 g. of methyl- H_2^3 iodide. This iodide, 4.35 mmoles, is again heated, in the manner described above, with 1.116 g. (3.81 mmoles) of 3,6-diacetamidoacridine in a similar vessel with a volume of 9 ml. The solid product of the second methylation weighs 1.641 g., which corresponds to 97.2% methylation.

(b) 3,6-Diamino-10-methyl- H_2^3 -acridinium Chloride Hydrochloride, (H_2^3 -Acriflavine Hydrochloride). The total methylated product from the above reaction, 8.323 g., is transferred into a flask with two necks, one of which contains a sintered glass filter. In an atmosphere of nitrogen, the solid is dissolved in 450 ml. of hot, dilute (1:10) hydrochloric acid, and the resulting solution is filtered. 3,6-Diamino-10-methyl- H_2^3 -acridinium chloride hydrochloride is precipitated by the addition of 550 ml. of concentrated hydrochloric acid and subsequent refrigeration of the solution. After the product is collected by filtration and dried *in vacuo*, the yield is 5.088 g. (89.0% based on 3,6-diacetamidoacridine) (Note 5).

B. Notes

1. The larger arm of the vessel was constructed of 15 mm. o.d. tubing sealed to a standard-taper, ground-glass joint inner member; the other arm, 6 mm. o.d., was made from an extended, standard-taper, ground-glass joint inner member sealed at both ends. The vessel was constructed with a volume of 36 ml. such that the pressure of methyl iodide would be 3-4 atmospheres when methylation was complete.

2. Preparation of 3,6-diacetamidoacridine from 3,6-diaminoacridine sulfate is described by Smith, Wilzbach and Brown.

3. The use of methyl sulfate¹ or *p*-toluenesulfonate^{2,3} in nitrobenzene for the methylation was unsatisfactory because the reaction is incomplete and separation of nonmethylated material (proflavine) from the product is difficult.^{3,4,5}

4. The extent of the reaction was estimated by the decrease in volume of the liquid methyl iodide.

5. The product formed a picrate melting at 245–246°; the melting point reported⁶ for the picrate of acriflavine is 244°. Analysis of the product by filter paper partition chromatography, using the method of Lederer,⁷ which was modified by developing the chromatograph with octyl alcohol saturated with 1.5 N ammonium hydroxide, indicated the presence of 1% of 3,6-diaminoacridine dihydrochloride (proflavine dihydrochloride). The method was calibrated with known mixtures.

¹E. Grandmougin and K. Smirous, *Ber.*, 46, 3431 (1913).

²L. Benda, *ibid.*, 45, 1796 (1912).

³A. Albert and B. Ritchie, *J. Chem. Soc.*, 1943, 458.

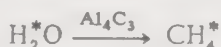
⁴J. Marshall, *Quart. J. Pharm. and Pharmacol.*, 1, 514 (1934).

⁵P. Gaillot, *Bull. Soc. chim. France*, [V] 1, 796 (1934).

⁶A. Bolliger, *Quart. J. Pharm. and Pharmacol.*, 13, 1 (1940).

⁷M. Lederer, *Anal. Chim. Acta*, 6, 267 (1952).

METHANE-H₃³



D. F. White, I. G. Campbell and P. R. Payne, *Nature*, 166, 628 (1950).

A. Procedure

The water-H₃³, 0.3 ml., is placed in a tube-like Pyrex reaction vessel. The water is frozen by immersion in a Dry Ice-acetone bath, approximately 2 g. of finely powdered aluminum carbide is added, and the vessel is evacuated to a pressure of less than 10⁻² mm. of mercury. The vessel is heated to 100° C. (Note 1) for 1 hour, since heating for shorter periods leaves droplets of water on the sides of the vessel (Note 2). The methane, as obtained, is impure, containing small amounts of hydrogen, oxygen, ammonia, hydrogen sulfide and unsaturated hydrocarbons.¹

B. Notes

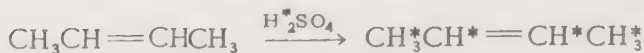
1. Water-H₂² reacts with aluminum carbide at a slow rate below 650° C.²

2. The following data show the relation between reaction time and activity of methane-H₃³.

Time in hours	Counts per second (Sample pressure 20 cm. Hg.)
1/6	23.7 ± 0.2
1/2	24.2 ± 0.2
1	24.0 ± 0.2
16	25.9 ± 0.2

¹W. M. Kemula, *Przemysl. Chem.*, 12, 411 (1928).

²H. C. Urey and D. Price, *J. Chem. Phys.*, 2, 300 (1934).

H³-2-BUTENE

T. D. Stewart and D. Harman, J. Am. Chem. Soc., 68, 1135 (1946).

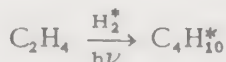
A. Procedure

Gaseous 2-butene is passed through a sintered glass bubbler and into 6 ml. of sulfuric acid-H₂³ (sp. act. 5.43×10^7 disintegrations per minute). The rate of passage is such that little absorption occurs; all of the effluent gas is collected in a liquid air-trap and amounts to about 2 ml. of liquid H³-2-butene (Notes 1 and 2).

B. Notes

1. The specific activity of the hydrogen obtained from this material was 6.1×10^5 disintegrations per minute and demonstrates not only a rapid exchange but also a reversible absorption of the alkene in sulfuric acid.

2. In an alkylation experiment, a solution composed of 0.48 mole of isobutane and 0.109 mole of 2-butene was added under pressure over a period of 15 minutes to 0.131 mole of sulfuric acid-H₂³, with vigorous stirring. The reaction chamber was a lead-lined cylindrical iron vessel held at 10°. A few minutes was allowed for completion of the reaction. After the excess of isobutane was allowed to evaporate from the reactor, the residual oil and acid layers were separated, and the former was washed with water and dried. The 20 ml. of alkylate was fractionated in a 20-plate column. Activity of the hydrogen, from each fraction collected, indicated extensive exchange (approximating random distribution) between the 2-butene and the acid, either prior to or during alkylation.

H³-BUTANE

L. Kaplan, J. Am. Chem. Soc., 76, 1448 (1954).

A. Procedure (Note 1)

Measured amounts of either acetylene or ethylene and hydrogen-H₂³, in excess (Note 2), are transferred into the evacuated reaction system. In the case of ethylene, reaction is continued until the pressure becomes

constant and all the ethylene is consumed (Note 3). After irradiation, the hydrocarbons are removed by circulating the gaseous mixture through a trap cooled to -195° . The mixture of hydrocarbons is then subjected to fractionation,^{1,2} at low pressure, through a series of traps maintained at successively lower temperatures (Note 4). The results of two typical runs are given in the following table.

TABLE XVI, 32
Mercury-photosensitized Tritiation and Polymerization

Reactants	Volume, cc. at S.T.P.	Activity ^a	Volume, cc. at S.T.P.	Activity ^a
C ₂ H ₂	26.2
C ₂ H ₄	25.5
H ₂	220	1.00	225	1.00
Products				
H ₂	202	1.03	210	1.01
C ₂ H ₂	3.5	0.32
C ₂ H ₆	1.3	0.97	2.45	0.76
C ₄ H ₁₀	0.75	1.5	8.0	0.64
C ₆ + C ₈ compounds	0.92	1.03	2.4	0.55

^aRelative molar specific activity; initial H₂³ mixture = 1.00

In the acetylene reaction, a large amount of polymer is formed, whereas, with ethylene no polymer is observed (Note 5).

B. Notes

1. The effect of mercury photosensitization on mixtures of acetylene and hydrogen-H₂³, and ethylene and hydrogen-H₂³ was studied in a reaction vessel consisting of a quartz tube which was irradiated with two 4-watt "Germicidal" lamps. The reaction mixture was circulated through the irradiated tube by means of a Toepler pump which also served as a source of mercury vapor. The volume of the system, which included a manometer, varied between 300 and 600 cc., depending on the position of mercury in the pump.

2. A large excess of hydrogen-H₂³ was used to minimize quenching by the hydrocarbons.

3. With acetylene, the reaction was stopped short of completion to permit determination of the tritium content of the residual acetylene.

4. Known mixtures of ethane, acetylene and butane were separated quantitatively by this procedure.

5. From material balances, the ratio of hydrogen to acetylene incorporated in the polymer was 0.6. The most unexpected result was the slowness of exchange between hydrogen- H^3 and acetylene, compared to the rates of hydrogenation and polymerization.

¹E. C. Ward, *Ind. Eng. Chem., Anal. Ed.*, **10**, 169 (1938).

²J. J. Savelli, W. D. Seyfried and B. M. Filbert, *ibid.*, **13**, 868 (1941).

H^3 -2-METHYLPROPANE

(H^3 -Isobutane)



T. M. Powell and E. B. Reid, *J. Am. Chem. Soc.*, **67**, 1020 (1945).

A. Procedure

The isomerization of butane (Note 1) is carried out at atmospheric pressure with temperatures in the range 107-121°. The feed butane, contained in a steel cylinder as a liquid under pressure, is vaporized through a reducing valve and conducted through a flow meter and a drier to a glass catalyst chamber inserted in a combustion tube-furnace. In addition to butane the reaction mixture contains hydrogen- H^3 chloride and hydrogen (Note 2). In a typical isomerization of butane at 121°, the catalyst is 20% aluminum chloride on alumina (Note 3). The feed stock is made up of butane (0.1 liquid volume/volume/hour), hydrogen- H^3 chloride (20% gas volume per cent of butane), and hydrogen (1 mole/mole of butane). Under these conditions 53.4% of the butane is converted to isobutane, which contains 11.7% of the activity originally present in the hydrogen- H^3 chloride (Notes 4 and 5).

B. Notes

1. According to Heldman¹⁻³ and others,⁴ pure, dry aluminum bromide and chloride are completely inert toward paraffin hydrocarbons, but addition of a hydrogen halide or a substance capable of producing one by reaction with the aluminum halide results in a catalytically active system. Under mild conditions, isomerization is the only reaction observed with the lower paraffins. The primary reaction is always the interchange of a methyl group with a hydrogen atom two (sometimes three) carbon atoms removed.

2. Hydrogen exchange was proportional to the amount of isomerization taking place; however, more exchange is obtained with hydrogen- H^3 chloride as the source of tritium than with hydrogen- H^3_2 as the source.

3. The catalysts, aluminum chloride on charcoal or alumina supports, were prepared in a hydrogen chloride atmosphere by impregnating the 8-14 mesh supports with aluminum chloride at 316° in a closed system.

4. It is of interest that the unisomerized butane (42.59%) contains 6.7% of the original activity.

5. In accordance with the isomerization mechanism proposed by Powell and Reid, it appears quite likely that the tritium will reside on the tertiary carbon in the isobutane. During the isomerization, the 2-3 bond of butane becomes partially olefinic, and hydrogens adjacent to double bonds have been shown⁵ to be available for such exchanges with hydrogen.

C. Other Preparations

Very little synthetic work has been done with tritium, and the authors,⁶ in this instance, have given no experimental details; however, the following reaction offers tremendous possibilities in selectively labeling hydrocarbons with tritium. Butane-1- H^3 , butane-2- H^3 , 2-methylpropane-2- H^3 and 2-methylpropane-1- H^3 were selectively labeled by hydrolyzing the corresponding butylmagnesium bromide with acidic solutions of water- H^3 .

Isobutane has also been labeled by exchange with sulfuric acid- H^3 .⁷ Forty ml. of isobutane and 6 ml. of 100% sulfuric acid (specific activity 5.43×10^7 counts/min./mole) were stirred vigorously for 20 minutes in a lead-lined reactor. The specific activity of the hydrogen from a sample of H^3 -isobutane corresponded to 7.1% of random distribution of hydrogen atoms of the acid and the tertiary hydrogen atoms of the alkane.

¹J. D. Heldman, J. Am. Chem. Soc., 66, 1789 (1944).

²*Idem*, 66, 1786 (1944).

³P. A. Leighton and J. D. Heldman, *ibid.*, 65, 2276 (1943).

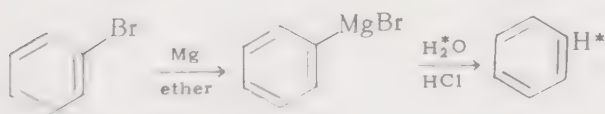
⁴G. Egloff, G. Hulla and V. I. Komarewsky, *Isomerization of Pure Hydrocarbons*, Reinhold Publishing Corporation, New York, 1942.

⁵K. Morikawa, N. R. Trenner and H. S. Taylor, J. Am. Chem. Soc., 59, 1103 (1937).

⁶T. M. Powell and E. B. Reid, J. Am. Chem. Soc., 67, 1020 (1945).

⁷T. D. Stewart and D. Harman, *ibid.*, 68, 1135 (1946).

H^3 -ARYL COMPOUNDS



A. Procedure (Note 1)

In the preparation of tracer amounts of aromatic tritium compounds some of the laborious details of the methods referred to in Note 1 are avoided without excessive impairment of yields. The filtered Grignard solution is poured into another portion of dry ether containing less than an equivalent amount of hydrochloric acid- H^3 (Note 2). During this addition, which is carried out rapidly with vigorous agitation, external cooling and a reflux condenser are necessary. After the acid- H^3 has reacted, the decomposition is completed with hydrochloric acid. The ether solution is then washed with water, sodium carbonate solution, and water, dried over calcium chloride and distilled through a column.

When the product is benzene or toluene, the distillation is interrupted when the temperature of the distillate reaches 35° , and the remainder of the ether is removed by extraction with two portions of 60% perchloric acid (Note 3). If the two layers are difficult to separate sharply, the aqueous phase is shaken with some of the corresponding nonisotopic hydrocarbon which is added to the main portion. The organic liquid is then washed with water, sodium carbonate solution and water, and dried with calcium chloride. The benzene or toluene is finally distilled twice from sodium through a small Podbielniak column at atmospheric pressure.

In the case of toluene- α - H^3 , benzyl chloride is the starting material; in all other cases, the bromine compounds are used. In the usual experiment, 0.1 mole each of the halide and magnesium are used in the preparation of the compounds listed in Table XVI, 33 (Note 4).

TABLE XVI, 33.
 H^3 -Aryl Compounds

Starting compound	Product	B.p. or m.p.	Refractive index	Activity yield, %
Bromobenzene	benzene- H^3	78.5-79.8 $^\circ$	$n_D^{20.2}$ 1.5008	25
2-Bromotoluene	toluene-2- H^3	108-109 $^\circ$	$n_D^{20.3}$ 1.4963	31
3-Bromotoluene	toluene-3- H^3	109-109.5 $^\circ$	$n_D^{20.5}$ 1.4965
4-Bromotoluene	toluene-4- H^3	110.5-111.5 $^\circ$	$n_D^{20.6}$ 1.4963	36**
Benzyl chloride	toluene- α - H^3	108.7-109 $^\circ$	$n_D^{20.3}$ 1.4963*	21
1-Bromonaphthalene	naphthalene-1- H^3	101-102 $^\circ$ / 21 mm.	(m.p. 79-80.5 $^\circ$)	21
1,2-Dibromobenzene	1-bromobenzene-2- H^3	52-54 $^\circ$ / 19 mm.	$n_D^{18.3}$ 1.5602	11.2
1,3-Dibromobenzene	1-bromobenzene-3- H^3	45-47 $^\circ$ / 12-13 mm.	$n_D^{20.0}$ 1.5584	33
1,4-Dibromobenzene	1-bromobenzene-4- H^3	59-61 $^\circ$ / 33 mm.	$n_D^{19.4}$ 1.5593	28

* After dilution with inactive toluene.

** See Note 5.

B. Notes

1. Although use of the Grignard reaction appears to be the only practical means of introducing tritium into definite positions in aromatic nuclei, there are definite limitations imposed by various substituents, e.g., nitro groups.

One of the practical difficulties is caused by the hygroscopic nature of the basic magnesium halide formed in the decomposition when water- H_2^3 is added to the Grignard reagent. If the reaction is to be completed in this way, a large excess of water is necessary. Langseth and Klit¹ used hydrogen- H^2 chloride instead of water- H_2^2 for the decomposition and prepared all of the partly deuterated benzenes in good yield. Weldon and Wilson² prepared benzene- H_1^3 by decomposition of the dry Grignard compound with water- H_2^2 thus diminishing the amount of ether to be separated from the benzene.

2. This is water- H_2^3 in hydrochloric acid.

3. Perchloric acid does not cause hydrogen exchange.³

4. Experiments showed that no hydrogen exchange occurs as a result of catalytic influences in this procedure.

5. An appreciable amount of 4,4'-bitolyl was found in the distillation residue, m.p. $118-120^\circ$ from alcohol. Its specific activity was only 0.9% of that of the toluene-4- H_1^3 . This fact indicates little or no exchange in these reactions.

C. Other Preparations

Benzene- H_6^3 has been prepared⁴ by exchange with slightly more than 50 mole per cent sulfuric acid- H_2^3 . The amounts used were 4 ml. of concentrated sulfuric acid, 1 ml. of dilute water- H_2^3 and 12 ml. of benzene. With stirring at room temperature, 17% of the tritium was recovered in the benzene after 5 days.

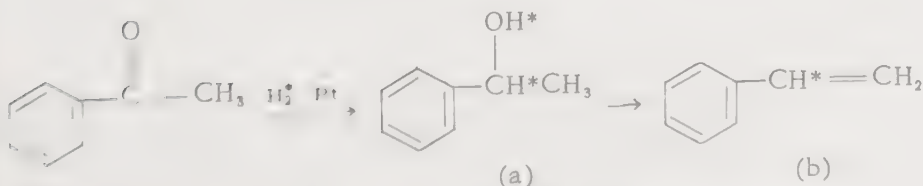
¹A. Langseth and A. Klit, K. Danske Videnskab. Selsk., Math.-fys. Medd., 15, (1937-38) No. 13.

²L. H. P. Weldon and C. L. Wilson, J. Chem. Soc., 1946, 235.

³A. P. Best and C. L. Wilson, *ibid.*, 1946, 239.

⁴L. Melander, Arkiv Kemi, 2, 260 (1950).

STYRENE- $\alpha\text{-H}^3$
(1-Phenylethylene-1- H^3)



I. A. Berstein, W. Bennett and M. Fields, J. Am. Chem. Soc., 74, 5763 (1952).

A. Procedure

(a) α -Methylbenzyl- α -H³ Alcohol-H³. The hydrogenation apparatus (Figure XVI, 13) is a Parr hydrogenation bottle held in a mechanical shaker and attached to a vacuum manifold. Also attached to the manifold are: a 350-ml. buret attached to a Toepler pump, a manometer, a vacuum line and a hydrogen-H₂³ ampoule. The hydrogenation bottle, containing 0.3 g. of platinum oxide catalyst and 5.0 ml. of acetophenone, and the entire vacuum system are evacuated with a water-aspirator. The bottle and apparatus are filled with tank hydrogen and evacuated several times. Finally, an atmosphere of hydrogen is left in the bottle, and the stopcock leading to the manifold is closed. With 15–20 mm. hydrogen pressure in the system and the buret filled with mercury from the Toepler pump, the hydrogen-H₂³ ampoule is opened at the break-seal. The hydrogen-H₂³ is pumped into the gas buret, and the ampoule is filled with tank hydrogen, which is also transferred to the buret. This is repeated several times until the buret contains about 150 ml. of gas at 1 atmosphere. The shaker is then started, and after this quantity of gas is used in the reduction, the filling process is repeated.

A total of 1158 ml. of hydrogen is used in the hydrogenation in 3.5 hours. The organic material is dissolved in ether and filtered to remove the catalyst. After most of the ether is distilled off, the remaining traces are removed *in vacuo* (Note 1).

(b) Styrene- α -H³, (1-Phenylethylene-1-H³). Into the flask of a distillation apparatus containing the α -methylbenzyl- α -H³ alcohol-H³ is added 10 mg. of picric acid and 5 mg. of *p*-toluenesulfonic acid. Also, 10 mg. of

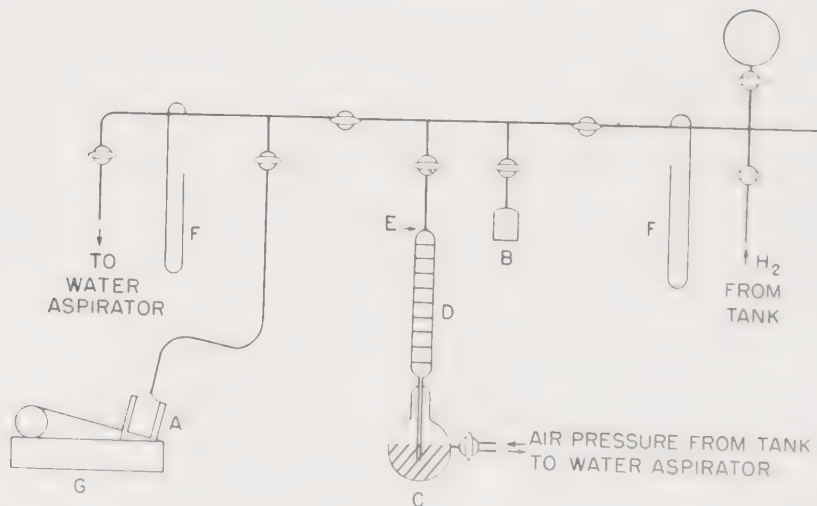


Fig. XVI, 13 Apparatus for preparation of styrene- α -H³ (I. A. Berstein, W. Bennett and M. Fields). A, hydrogenation bottle; B, hydrogen-H₂³ ampoule; C, Toepler pump; D, 350-ml. gas buret; E, fritted disk; F and F', manometers; G, shaking device.

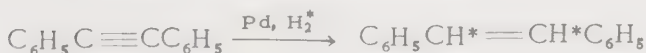
picric acid is placed in the receiver. With an oil-bath at 155–190°, the water and styrene distill at 42–68° (100 mm.). After drying the product, dissolved in ether, over magnesium sulfate, 3.54 g. (79%) of styrene- α -H³ is obtained by distillation; b.p. 62.5–63.5° (60 mm.), n_D^{27} 1.5409 (Note 2).

B. Notes

1. In a comparable nonisotopic experiment, α -methylbenzyl alcohol was isolated in 92% yield; b.p. 88° (13.5 mm.), $n_D^{25.5}$ 1.5260.

2. To determine whether any tritium had exchanged with hydrogen on the benzene ring, the styrene was oxidized to benzoic acid with permanganate. To ensure absence of tritium from the carboxyl group, the H³-benzoic acid was dissolved in 5% sodium hydroxide and precipitated by addition of sulfuric acid. This process was repeated three times. The benzoic acid, m.p. 122.8–123.0°, thus obtained, contained 9% of the total tritium in the styrene.

trans-STILBENE- α,β -H₂³ (*trans*-1,2-Diphenylethylene-H₂³)



I. A. Bernstein, W. Bennett, M. Fields and E. C. Farmer, *Nucleonics*, 11 (2), 64 (1953).

A. Procedure

A solution of 1 g. of diphenylacetylene in 7 ml. of dry benzene and 0.05 g. of 1% palladium-on-calcium carbonate catalyst are placed in a 250-ml. hydrogenation bottle. The hydrogenation is carried out with a 1-curie ampoule of hydrogen-H₂³ according to the procedure described for the preparation of styrene- α -H³ (Note 1). Following the hydrogenation, the mixture is filtered through Celite to separate the catalyst. The solvent is then distilled at 20 mm. with a bath at 50° (Note 2).

The liquid *cis*-stilbene- α,β -H₂³ obtained from the hydrogenation is transferred into a bomb tube, frozen in liquid nitrogen, degassed, refrozen, and sealed under vacuum. The tube is placed in a preheated furnace at 315° for 1.5 hours. On cooling, a mixture of white crystals and yellow solid results. The *trans*-stilbene- α,β -H₂³ is purified by chromatographic adsorption on alumina and elution with pentane; yield 0.67 g. (67%).

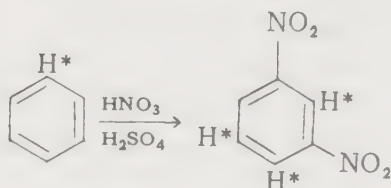
B. Notes

1. A diagram of the apparatus¹ is shown in Fig. XVI, 13. It is not necessary to first reduce the palladium catalyst.

2. Analysis of the benzene solvent showed no radioactivity and indicated that no exchange had occurred using this palladium catalyst.

¹I. A. Bernstein, W. Bennett and M. Fields, J. Am. Chem. Soc., 74, 5763 (1953).

H³-NITROARYL COMPOUNDS



L. Melander, Arkiv Kemi, 2, 270 (1950).

A. Procedure

The following general procedure is used in the nitration of tritium-labeled benzene and toluene (Note 1).

To 5-8 ml. of a mixture (1:2 by volume) of concentrated nitric acid (sp. gr. 1.40) and concentrated sulfuric acid (96%) is added 0.50 ml. of benzene-H³. The resulting mixture is shaken for 4 minutes in a tightly stoppered flask. Then the mixture is poured onto crushed ice, with stirring. The precipitated, crystalline solid is collected by filtration, washed with water and recrystallized from alcohol.

The compounds prepared by the above general procedure, with specific experimental details, are given in the following table. Yields are determined by the isotopic dilution method (Note 2).

The possibility of hydrogen exchange is checked by nitrating inactive starting compound with an acid mixture containing a small amount of water-H₂³. Toluene and naphthalene do not exchange hydrogen to any significant degree, under these experimental conditions (Note 3).

B. Notes

1. For the purpose of the work described, i.e., determination of the ratio of the rates of substitution of protium and tritium, it was desirable that the yields of the reactions should be high and, if possible, lead to a single product. Since many monosubstitutions of ortho-para-directing benzene derivatives do not conform to this requirement, e.g., nitration of toluene or bromobenzene, the reaction was carried further to the disubstituted derivatives.

2. An inactive preparation of each compound was made, and to the crude product was added a weighed amount of tritium-containing molecules of the same species, with a known specific activity. After recrystallization of the mixture, the specific activity of the product was

TABLE XVI, 34

Nitration of H^3 -Aromatic Hydrocarbons

Starting Compound		Mixed acids ml.	Water, ml.	Product	M.p. (uncor.)	Yield,	
Name	ml.					%	Y*/Yo
Benzene- H^3	0.50	8	0.03	1,3-dinitro- benzene- 2,4,5- $H^3_{1/3}$	86-88°	79-86	0.664
Toluene-2- H^3	0.50	10	0.06	2,4-dinitro- toluene- 6- H^3	68.5-69.5°	75-80	0.514**
Toluene-3- H^3	0.50	10	0.06	2,4-dinitro- toluene- 3,5- $H^3_{1/2}$	68-69°	75-80	0.990
Toluene-4- H^3	0.50	10	0.06	2,4-dinitro- toluene	67.5-68.5°	75-80	0.022
Toluene- α - H^3_1	0.50	10	0.06	2,4-dinitro- toluene- α - H^3_1	69-70°	75-80	0.984
Naphthalene- 1- H^3	0.50 g.	5-10***	2%	1-nitronaph- thalene- 4,5,8- $H^3_{1/3}$	55-58°	93	0.766**

*Y = ratio of isotopic to nonisotopic molecules in product;

Yo = same ratio in starting material.

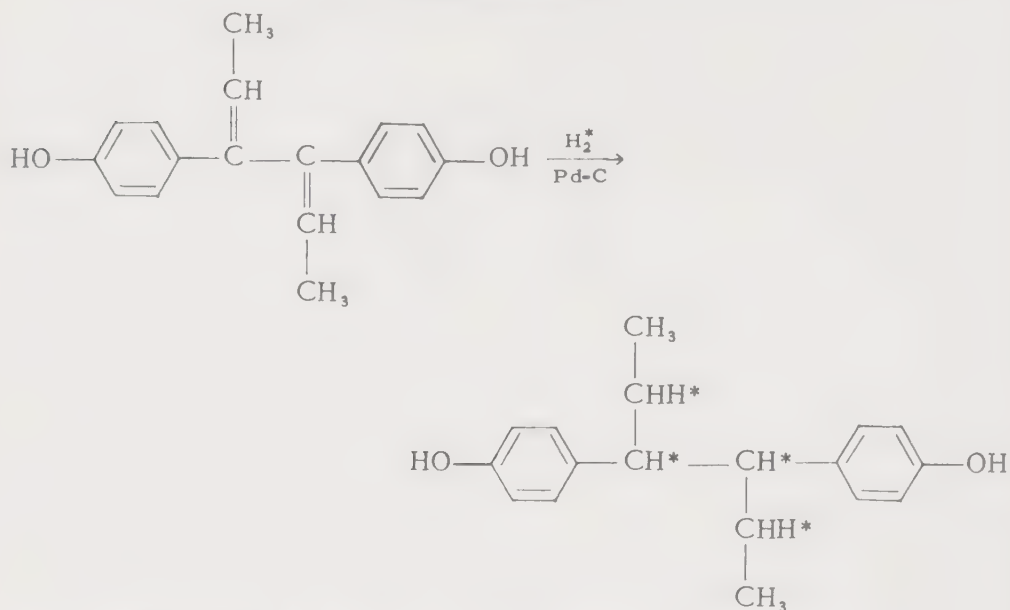
**Mean value from several experiments.

***Concentrated nitric acid only.

determined and the yield calculated. For example, if 100 mg. of 1,3-dinitrobenzene-2,4,5- $H^3_{1/3}$, specific activity 16×10^3 d./min./mg., is recrystallized with a crude product, and the final specific activity is 2×10^3 d./min./mg., then the yield of inactive compound is $\left(\frac{16}{2} - 1\right) 100 = 700$ mg.

3. Benzene was not checked, since no hydrogen exchange was expected under the experimental conditions employed, and none was found in the case of toluene.

4,4'-[1,2-BIS(ETHYL-1-H³)ETHYLENE-H₂³]DIPHENOL
(H₄³-Hexestrol)



D. L. Williams and A. R. Ronzio, J. Am. Chem. Soc., 72, 5787 (1950).

A. Procedure

The labeling of hexestrol with tritium is carried out in an all-glass vacuum apparatus (see Figure XVI, 14) which is sealed off from the main manifold after evacuation. A U-tube, A, connected to two break-off sections, Y and Z, and containing 500 mc. of hydrogen-H₂³ mixed with hydrogen (5 ml. total), is sealed into position as part of a small vacuum manifold which is connected to a 100-ml. gas buret, D. A small reaction flask, B, containing 0.503 g. of dienestrol, 0.054 g. of catalyst (Note 1), 5 ml. of acetone and a magnetic stirrer, is cooled in liquid nitrogen and then sealed to the manifold (Note 2). With the contents of the flask still frozen, the entire system is evacuated to a pressure of 3-5 microns and, after testing for leakage, the reaction system is isolated from the main manifold by sealing off the connecting tubing at G and F. When the solvent is melted, break-off section Y is broken, and the hydrogen-H₂³ is allowed to react with the well stirred reaction mixture for 45 minutes. In the meantime, the gas buret is filled with hydrogen. With the stirrer at rest and with the leveling bulb, K, raised to maintain a positive pressure in the system, the second break-off section, Z, is broken. At this point the initial reading of the volume of hydrogen is taken. When stirring is resumed, the flow of hydrogen serves to flush most of the tritium out of the U-tube. Progress of the reaction is followed by the decrease in volume of hydrogen.

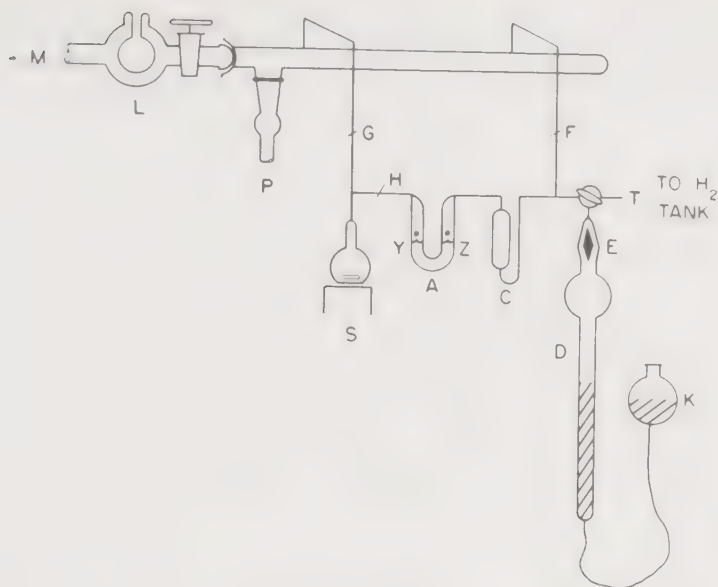


Fig. XVI, 14 Apparatus for the preparation of 4,4'-[1,2-bis(ethyl-1- H^3)ethylene- H_2^3]diphenol (D. L. Williams and A. R. Ronzio). A, U-tube containing tritium; B, hydrogenation flask; C, U-trap; D, 100-ml. gas buret; E, valve to prevent mercury from leaving buret; F, G, and H, constrictions for sealing off; K, mercury reservoir; L, liquid-air back diffusion trap; M, to pumps; P, to Pirani gage; S, magnetic stirrer; Y and Z, break seals.

After reaction is complete, the leveling bulb is lowered and the solvent is distilled into tube C, using liquid nitrogen as coolant. When all the solvent is distilled off, the reaction flask, B, is removed by sealing off the neck. The product is isolated by redissolving it in dry acetone and filtering the solution through a thin bed of diatomaceous earth and decolorizing carbon. The flask and filtering medium are washed with acetone (total volume about 20 ml.). Evaporation of the filtrate under reduced pressure gives a yield of 0.5946 g. of crude product (116.2%) (Note 3).

Separation of H_4^3 -*meso*-hexestrol and H_4^3 -DL-hexestrol (isohexestrol) is effected by recrystallization of the crude product from 30 ml. of benzene (Note 4) at room temperature and then at 6–8° (2 hours). The mother liquor is removed with a filter stick. The colorless crystals, after drying in a vacuum, weigh 0.386 g. (73%) and melt at 184–185° (Note 5).

Evaporation of the mother liquor to dryness leaves a colorless residue which is recrystallized from 5 ml. of benzene to obtain about 0.034 g. (6%) of material melting at 160–164°, apparently a mixture of H_4^3 -hexestrol and H_4^3 -isohexestrol. The second mother liquor is evaporated to a volume of 1.5 ml. and is cooled to 6° for 1 hour. The mother liquor is removed as before, and the colorless needles, washed with 5 ml. of benzene and dried, weigh about 0.142 g. (28%) and melt at 126–127°. The mother

liquor from these crystals, upon evaporation to dryness, yields about 0.03 g. of impure H_4^3 -isohexestrol, m.p. 124–125°.

B. Notes

1. The catalyst was 10% palladium-on-carbon.
2. With suitable adjustment of the volumes of the reaction flask and the tritium reservoir, any desired amount of product, ranging from a few mg. to several mmoles, can be prepared in this apparatus. The volume of solvent need not be reduced to the point where stirring is not possible. Since the use of extremely high activities, such as those attainable with pure hydrogen- H_2^3 , appear impractical due to radiation-induced decomposition or polymerization of the product (see succinic- H_4^3 acid), it is quite practical and often desirable to complete the reaction with ordinary hydrogen.
3. When either absolute ethanol or acetone was used as solvent, the weight yield was in excess of 100%. The excess weight corresponded closely to 1 mole of solvent per mole of product and was not removed by drying under vacuum. Heating the material at 100° caused sublimation and loss of product.
4. The solubility data¹ for pure hexestrol in benzene in the temperature range 15–16° indicates that a ratio of 5 ml. of benzene per 100 mg. of hexestrol should give a 90% return of product upon recrystallization.
5. Dodds, *et al.*,² who first isolated hexestrol, m.p. 184–185°, from the demethylation products from anethole, also isolated an isomer melting at 128°. Wessely and Welleba³ have shown the latter compound to be the racemic form and assigned the *meso*-structure to hexestrol.
6. According to Lawson⁴ palladium-black catalyst, prepared by the method of Heilbron,⁵ affords a product consisting predominantly of *meso*-hexestrol.

C. Other Preparations

A micro-scale preparation of 4,4'-[1,2-bis(ethyl-1- H_1^3)ethylene- H_2^3]diphenol (H_4^3 -*meso*-hexestrol) has been described by Glascock.⁶ The high vacuum apparatus used was designed for hydrogenations using about 1 ml. of hydrogen- H_2^3 (see octadecanoic-9,10- H_2^3 acid). Hydrogenation of 7.7 mg. of dienestrol in about 0.05 ml. of dioxane, with 4.2 mg. of palladium-black catalyst (Note 6) and 1.42 ml. of hydrogen- H_2^3 , gave 6.1 mg. (78.2%) of product, m.p. 176–178°, after two recrystallizations from benzene-ligroin.

The synthesis of 4,4'-[1,2-bis(ethyl-1- H_1^3)ethylene]diphenol, (H_2^3 -hexestrol), in about 10% over-all yield based on water- H_2^3 has been developed by Lacassagne and co-workers.⁷ *p*-(1-Chloropropyl-2- H_1^3)anisole, prepared from anethole and hydrogen- H^3 chloride, is condensed⁸ in aqueous

suspension with iron powder at 90° to obtain α, α' -bis(ethyl-1- H^3)-4,4-dimethoxybibenzyl. The latter compound is demethylated by the action of pyridinium chloride to obtain H^3_2 -hexestrol, m.p. $184-185^\circ$ from benzene.

¹J. Cheymol and A. Carayon-Gentil, Bull. soc. chim. biol., 28, 136 (1946); through Chem. Abstracts, 41, 3518 (1947).

²N. R. Campbell, E. C. Dodds and W. Lawson, Nature, 142, 1121 (1938).

³F. V. Wessely and H. Welleba, Ber., 74, 777 (1941).

⁴W. Lawson, private communication to R. F. Glascock.

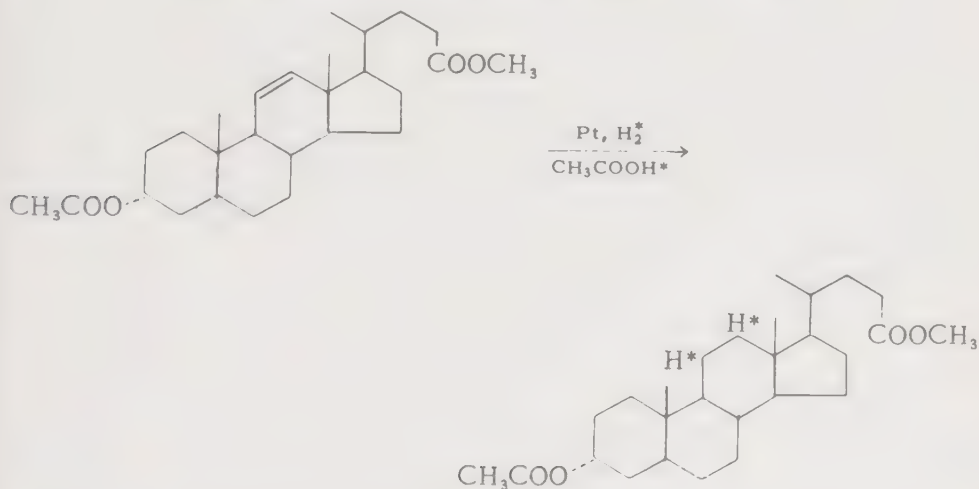
⁵I. M. Heilbron, W. A. Sexton and F. S. Spring, J. Chem. Soc., 1929, 926.

⁶R. F. Glascock, *Isotopic Gas Analysis for Biochemists*, Academic Press Inc., New York, 1954, p. 222.

⁷A. Lacassagne, N. P. Buu-Hoï, N. D. Xuong, F. Zajdela and B. Eckert, Compt. rend., 235, 40 (1952).

⁸A. Lacassagne, N. P. Buu-Hoï, A. Chamorro, N. D. Xuong and N. Hoán, *ibid.*, 231, 1384 (1950).

METHYL 3 α -ACETOXYCHOLANATE-11,12- H^3_2



M. L. Eidinoff, J. E. Knoll, D. K. Fukushima and T. F. Gallagher, J. Am. Chem. Soc., 74, 5280 (1952).

A. Procedure

A mixture of 21 g. of acetic acid- H^3 , 0.5 g. of methyl 3 α -acetoxy-11-cholelate and 0.04 g. of platinum oxide catalyst is shaken for 2.5 hours. Then approximately 0.006 mole of hydrogen- H^3_2 is added (Note 1) which is about a 4.5-fold excess, and the reaction is complete in about 13 minutes. The reaction mixture is cooled in Dry Ice until the hydrogen is removed; then the acetic acid- H^3 is removed by distillation *in vacuo*. Several successive quantities of ordinary acetic acid are added to the

product and also removed *in vacuo*. The product is dissolved in acetone, and the solution is filtered to remove catalyst. After two recrystallizations from petroleum ether, the methyl 3 α -acetoxycholanate-11,12- H_2^3 has the following properties: m.p. 134–135°; $[\alpha]_D^{25} + 48.1^\circ$ (acetone), $[\alpha]_D^{15} 48.4^\circ$ (Notes 2, 3 and 4).

B. Notes

1. Since measurement of isotopic fractionation during the hydrogenation was the purpose of the experiment, the reaction gas was first isotopically equilibrated with a large excess of acetic acid of isotopic composition equal to that used in the subsequent hydrogenation.

2. Eidinoff and Knoll¹ prepared methyl 3 α -acetoxycholanate-11,12- H_2^3 by a similar procedure and reported as m.p. 134.5°.

3. Using the average value from two experiments, it was found that protium was preferentially incorporated into the product by a factor of 5.42 ($\pm 2\%$), relative to the isotopic composition of the acetic acid carboxyl hydrogen. Other experiments have shown that isotopic composition of the acetic acid carboxyl hydrogen is of principal importance in determining the isotope ratios for the hydrogen atoms incorporated during the reduction. When tank hydrogen containing no hydrogen- H_2^3 was used in the reduction, the factor increased only slightly to 6.03.

4. It was also found that tritium was preferentially incorporated into the steroid by a factor of 1.26, relative to the composition of the hydrogen gas phase.

¹M. L. Eidinoff and J. E. Knoll, *Science*, 112, 250 (1950).

H^3 -CHOLESTEROL

R. G. Gould, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure (Note 1)

(a) *H³-Cholesterol*. To a mixture of 18 g. of cholesteryl acetate, dissolved in 50 ml. of glacial acetic acid, and 1.0 g. of prerduced platinum oxide catalyst¹ (Note 2) is added 10 ml. of water- H_2^3 . With the mixture frozen in a Dry Ice-acetone or liquid nitrogen-bath, the flask is evacuated and sealed. Using a calibrated heating mantle, the mixture is shaken at 127° for 3 days. Then, the flask is cooled in liquid nitrogen, opened and immediately attached to a second reaction flask. The solvent mixture is distilled into the second flask under vacuum (Note 3). The residue is extracted with chloroform, which is filtered to remove the catalyst. The catalyst is washed with chloroform, and the combined filtrates are evaporated to dryness *in vacuo*. The solvent is collected in a

liquid nitrogen-cooled trap. The crude ester is dissolved in alcohol and hydrolyzed with alcoholic potassium hydroxide. H^3 -Cholesterol is crystallized by concentrating and cooling the alcoholic solution (Notes 4 and 5).

(b) 3β -Cholestanol-5,6- H_3^3 . To a mixture of 20 g. of cholesterol, dissolved in 300 ml. of glacial acetic acid, and 0.5 g. of reduced platinum oxide catalyst is added 10 ml. of water- H_2^3 . The mixture is then shaken with hydrogen at 10-15 lbs. pressure. When hydrogenation is complete, the solvent is distilled off *in vacuo* for reuse. The product is dissolved in alcohol and filtered to remove the catalyst. Alcoholic potassium hydroxide is added, and the solution is refluxed for 8 hours. After cooling the solution, the crystalline product is precipitated by the addition of an equal volume of water. It is collected, washed with water and again heated with alcoholic alkali for 8 hours (Note 6). The solution is cooled diluted to 70% alcohol with water and extracted with petroleum ether. The petroleum ether solution is washed with water and evaporated to obtain a nearly quantitative yield of 3β -cholestanol-5,6- H_3^3 (Note 7).

(c) H^3 - 3β -Cholestanol (labeled in side chain). H^3 -Cholesterol labeled by exchange is hydrogenated with ordinary hydrogen in the presence of platinum oxide catalyst. According to the work of Gallagher² with H^2 -cholesterol, the isotope at the 6 position exchanges with hydrogen of the medium; therefore, the resulting H^3 - 3β -cholestanol should be labeled only in the side chain isopropyl group.

(d) H^3 -7-Cholesten- 3β -ol. 5,7-Cholestadien- 3β -yl acetate is hydrogenated with ordinary hydrogen and platinum oxide catalyst in an ether medium containing a small amount of water- H_2^3 .

(e) H^3 - β -Sitosterol. β -Sitosterol is exchanged with water- H_2^3 according to the procedure described for H^3 -cholesterol.

B. Notes

1. The procedure is essentially that described by Gallagher^{2,3} and by Bloch⁴ for the preparation of deuterium-labeled cholesterol. According to Gallagher,³ acetylation of sterols results in less destruction without materially altering the exchange.

2. The catalyst is prereduced with hydrogen before addition of the tritium water.

3. The solvent may then be used again in another exchange with cholesterol.

4. Kritchevsky,⁵ who followed the procedure of Bloch⁴ with slight modification, purified the H^3 -cholesterol by chromatography on an alumina column. The cholesterol was eluted with benzene and benzene-ether (1:1 ratio). The material eluted (56%) was further purified by preparation of the dibromide and then several recrystallizations from

acetone; m.p. 147°, $[\alpha]_D^{25}$ -39.3° (c, 0.4500 in chloroform).

5. According to the degradative study made by Fukushima and Gallagher,² on H³-cholesterol, prepared in the same manner by exchange, about 40% of the tritium should be at C-6, about 3% at C-3, and the remainder, approximately 50%, should be distributed at or among C-24, C-25, C-26, and C-27.

6. This treatment removes tritium from the hydroxyl group.

7. Exchange of tritium for both the hydrogens on C-6 occurs in addition to the single exchange at C-5.

C. Other Preparations

H³-Cholesterol has been prepared⁵ by catalytic exchange⁴ between an acetic acid-water-H₂³ medium and cholesterol (see Note 4).

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

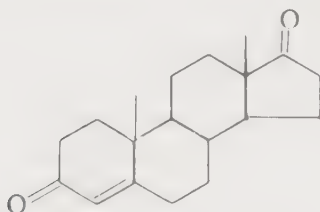
²D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, 198, 861 (1952).

³*Idem.*, *Federation Proc.*, 9, 174 (1950).

⁴K. Bloch and D. Rittenberg, *J. Biol. Chem.*, 148, 505 (1943).

⁵D. Kritchevsky, M. W. Biggs and N. K. Freeman, U.S. Atomic Energy Comm. Report UCRL-644, *Nuclear Sci. Abstr.*, 4, 3414 (1950).

H³-4-ANDROSTENE-3,17-DIONE



D. K. Fukushima and T. F. Gallagher, *J. Biol. Chem.*, 198, 871 (1952).

A. Procedure

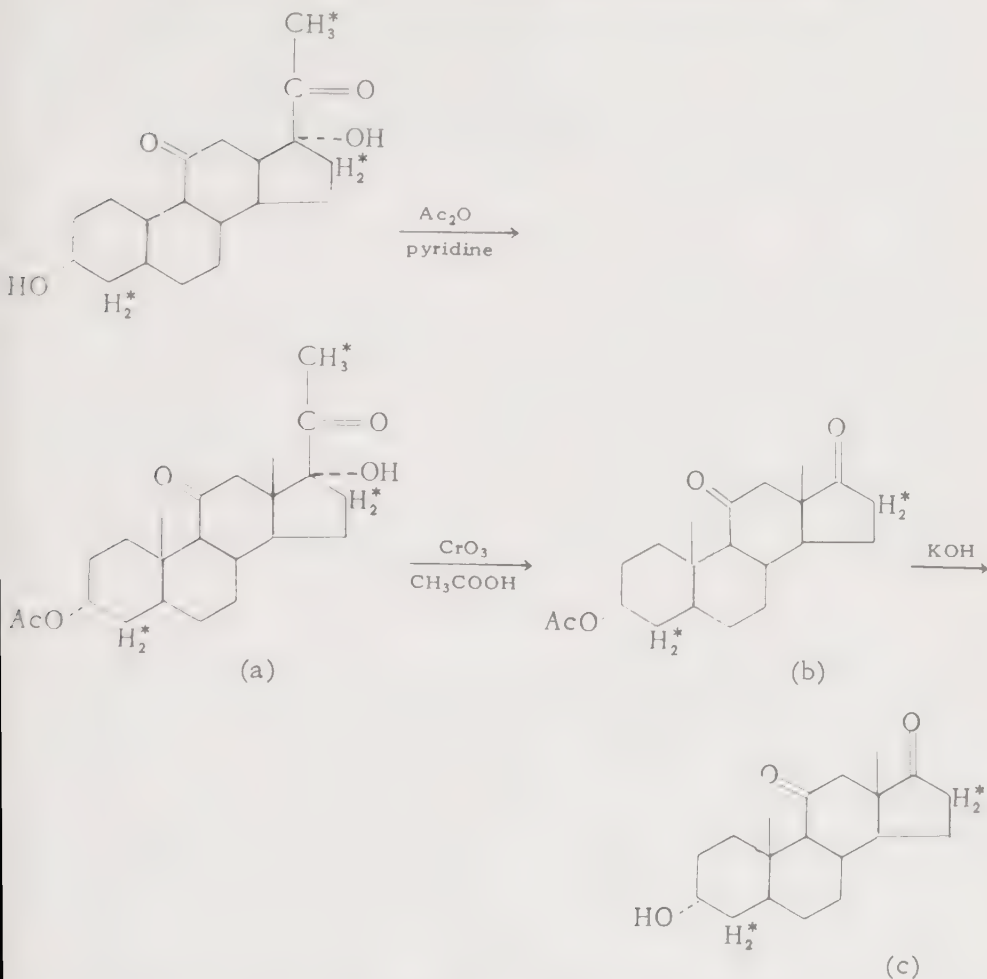
A mixture of 1.00 g. of 4-androstene-3,17-dione, 24 ml. of 70% acetic acid in tritium-enriched water and 500 mg. of platinum catalyst¹ (Note 1), is agitated at 150° for 2 days. After removal of the catalyst and distillation of the solvent *in vacuo*, the product is purified by chromatography on alumina (Note 2).

B. Notes

1. The catalyst was prereduced with tritium-enriched hydrogen gas.

2. Purified H³-4-androstene-3,17-dione was then refluxed with aqueous methanol under nitrogen. The tritium content of the product (about 2 atom per cent) changed very little after the first 4 hours.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 463.

3 α -HYDROXYETIOCHOLANE-11,17-DIONE-4,16- H_4^3 

D. K. Fukushima, T. H. Kritchevsky, M. L. Eidinoff and T. F. Gallagher, J. Am. Chem. Soc., 74, 487 (1952).

A. Procedure

(a) 3 α -Acetoxy-17 α -hydroxypregnane-11,20-dione-4,16,21- H_7^3 . 3 α ,17 α -Dihydroxypregnane-11,20-dione-4,16,21- H_7^3 is treated with acetic anhydride and pyridine at room temperature. After removal of pyridine and excess acetic anhydride, the product is recrystallized from petroleum ether-acetone (Note 1).

(b) 3 α -Acetoxyetiocholan-11,17-dione-4,16- H_4^3 . A solution of 1.6 g. of chromium trioxide in 60 ml. of 90% acetic acid is added to a solution of 0.850 g. of 3 α -acetoxy-17 α -hydroxypregnane-11,20-dione-4,16,21- H_7^3 in 20 ml. of acetic acid. After 1.5 hours at room temperature, the product is recrystallized from methanol and then ethyl acetate-petroleum ether, m.p. 159-161°. There is no depression in the melting point of the product admixed with an authentic sample (Note 2).

(c) *3 α -Hydroxyetiocholane-11,17-dione-4,16- H_4^3* . A solution of 0.350 g. of *3 α -acetoxyetiocholane-11,17-dione-4,16- H_4^3* is heated under reflux for 2 hours with 10 ml. of methanol, 3 ml. of water and 10 ml. of 5% methanolic potassium hydroxide. Purification of the product by chromatography on alumina and recrystallization from acetone-petroleum ether gives *3 α -hydroxyetiocholane-11,17-dione-4,16- H_4^3* , m.p. 187–188.5°.

B. Notes

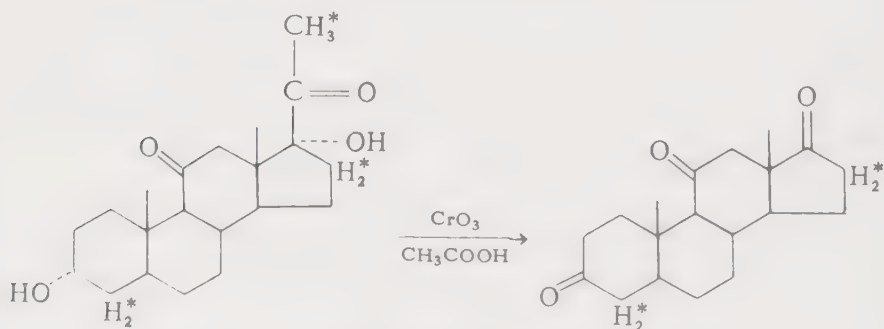
1. The nonactive material, prepared¹ in the same manner, melted at 202–204°; $[\alpha]_D^{30} + 81^\circ$ (acetone). The literature² records m.p. 208–209°; $[\alpha]_D^{30} + 84^\circ$ (acetone).

2. The location of the stably bound tritium was established in a degradative study of the location of tritium in cortisone-4,16,21- H_3^3 acetate.

¹T. H. Kritchevsky, D. L. Garmaise and T. F. Gallagher, J. Am. Chem. Soc., 74, 483 (1952).

²L. H. Sarett, J. Am. Chem. Soc., 70, 1454 (1948).

ETIOCHOLANE-3,11,17-TRIONE-4,16- H_4^3



D. K. Fukushima, T. H. Kritchevsky, M. L. Eidinoff and T. F. Gallagher, J. Am. Chem. Soc., 74, 487 (1952).

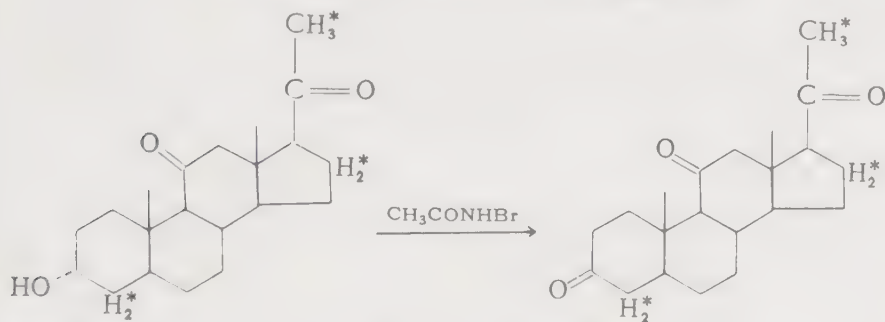
Procedure

A solution of 0.900 g. of *3 α ,17 α -dihydroxypregnane-11,20-dione-4,16,21- H_3^3* in 25 ml. of acetic acid is treated with a solution of 1.9 g. of chromium trioxide in 65 ml. of 90% acetic acid.

After 1 hour at room temperature, 0.220 g. of an oil is isolated and heated under reflux for 2 hours with 10 ml. of methanol, 3 ml. of water and 10 ml. of 5% methanolic potassium hydroxide. The product is chromatographed on alumina and recrystallized from ether-petroleum ether to obtain *etiocholane-3,11,17-trione-4,16- H_4^3* , m.p. 130–131°, with no depression of the melting point on admixture with an authentic sample.

This is one of the products obtained in the degradation of 3α - 17α -dihydroxypregnane-11,20-dione-4,16,21- H^3 to establish the location of stably bound tritium in cortisone-4,16,21- H^3 acetate.

PREGNANE-3,11,20-TRIONE-4,16,21- H^3



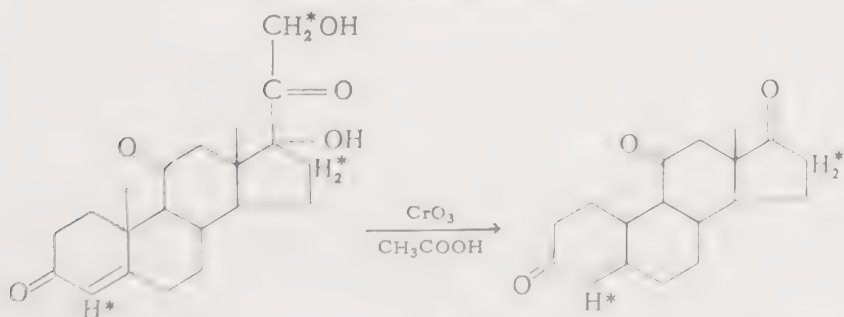
D. K. Fukushima, T. H. Kritchevsky, M. L. Eidinoff and T. F. Gallagher, J. Am. Chem. Soc., 74, 487 (1952).

Procedure

To a solution of 1.07 g. of 3α -hydroxypregnane-11,20-dione-4,16,21- H^3 in 25 ml. of *t*-butyl alcohol and 1.5 ml. of water is added 1.1 g. of *N*-bromoacetamide. After the solution is stored in the refrigerator for 18 hours, it is diluted with ethyl acetate and washed with 5% sodium hydroxide solution and with water. The solvents are removed, and the residue is twice recrystallized from petroleum ether-acetone. The pregnane-3,11,20-trione-4,16,21- H^3 melts at $151-154^\circ$. The melting point indicates a mixture with the 17α -epimer.

This is one of the products obtained in the degradative study made to establish the location of stably bound tritium in cortisone-4,16,21- H^3 acetate.

4-ANDROSTENE-3,11,17-TRIONE-4,16- H^3
(Adrenosterone-4,16- H^3)



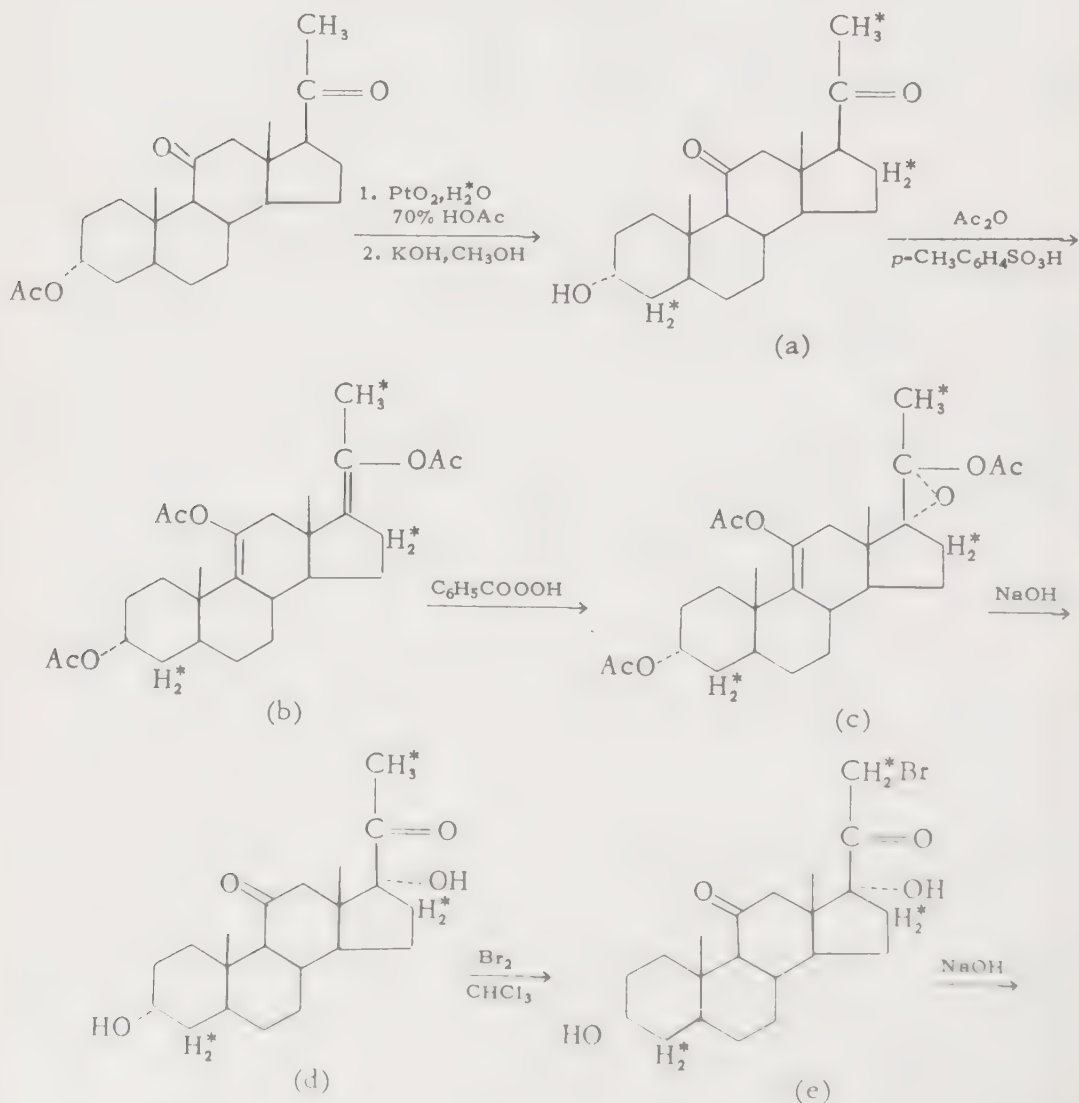
D. K. Fukushima, T. H. Kritchevsky, M. L. Eidinoff and T. F. Gallagher, J. Am. Chem. Soc., 74, 487 (1952).

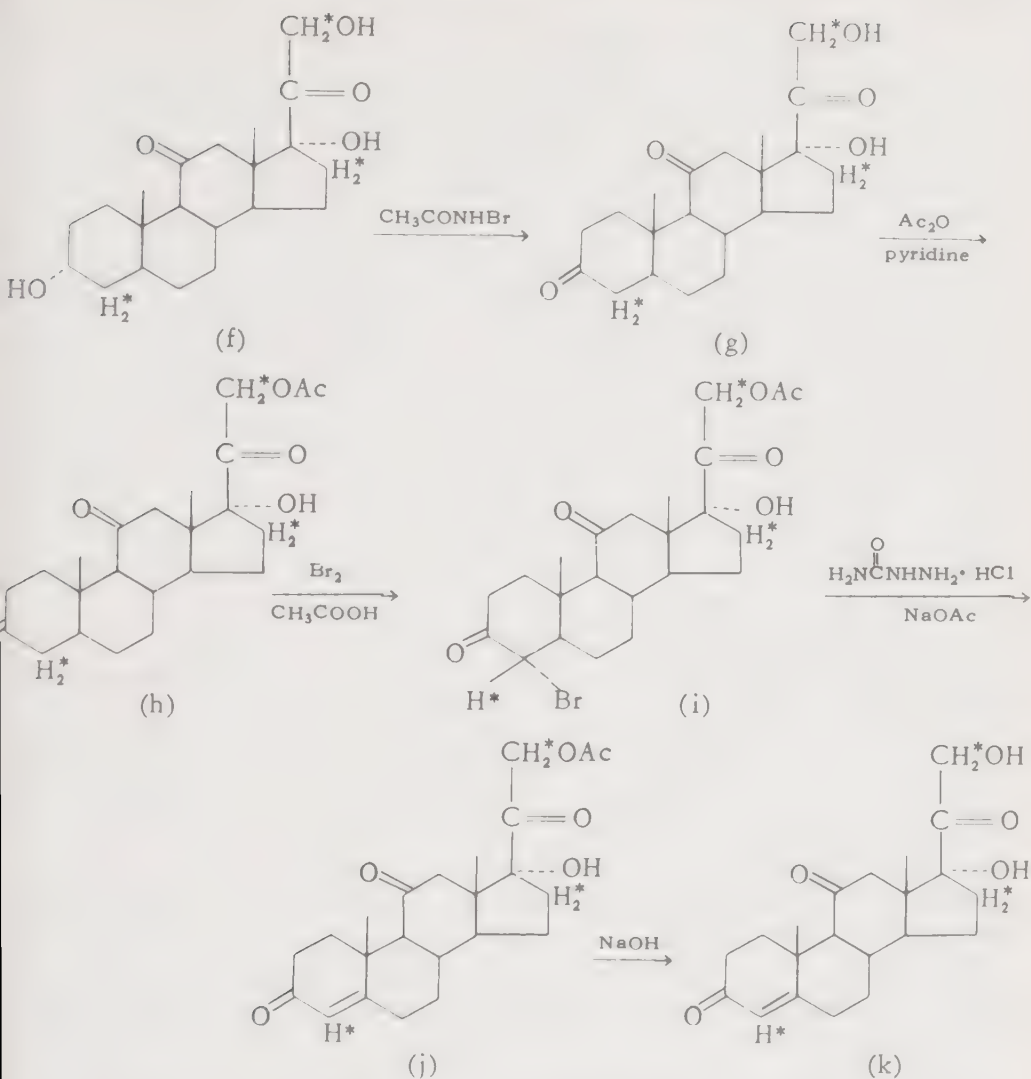
Procedure

To the residue of cortisone-4,16,21- H_3^3 from 2.00 g. of the acetate, dissolved in 75 ml. of glacial acetic acid, is added 75 ml. of a 1% solution of chromium trioxide in glacial acetic acid. The mixture is stored at room temperature for 17 hours. After recrystallization from methanol, the adrenosterone-4,16- H_3^3 melts at 222–223°.

This is one of the products obtained in a degradative study of the location of stably bound tritium in cortisone-4,16,21- H_3^3 acetate.

17 α ,21-DIHYDROXY-4-PREGNENE-3,11,20-TRIONE-4,16,21- H_3^3 (Cortisone-4,16,21- H_3^3)





D. K. Fukushima, T. H. Kritchevsky, M. L. Eidinoff and T. F. Gallagher, J. Am. Chem. Soc., 74, 487 (1952).

A. Procedure (Note 1)

(a) *3 α -Hydroxypregnane-11,20-dione-4,16,21- H_7^3* . A suspension of 0.250 g. of Adams platinum oxide catalyst in 70% acetic acid and tritium-enriched water is reduced with a tritium-hydrogen mixture. To the catalyst suspension is added 0.500 g. of 3 α -acetoxypregnane-11,20-dione and 9 ml. of tritium-enriched 70% acetic acid. The reaction mixture is frozen and evacuated, and the flask is sealed. It is then rotated at 150° for 2 days. The catalyst is removed by filtration, and the solvent is recovered in a closed vacuum system (Note 2). The residues from three runs are combined and heated under reflux for 2 hours, in a nitrogen

atmosphere, with 75 ml. of methanol, 15 ml. of water, and 60 ml. of 5% methanolic potassium hydroxide (Note 3). After neutralization of the base, 0.800 g. of nonisotopic 3α -hydroxypregnane-11,20-dione is added, and the diluted mixture is chromatographed on silica gel. Elution with acetone-ether mixtures (0.3% to 20%) yields 1.72 g. of crude 3α -hydroxypregnane-11,20-dione-4,16,21- H^3 .

(b) $3\alpha, 11, 20$ -Triacetox-9,17-pregnadiene-4,16,21- H^3 . The dienol triacetate is prepared essentially according to the procedure of Marshall.¹ A solution of 2 mmoles of the ketone and 2 mmoles of *p*-toluenesulfonic acid in 75 ml. of acetic anhydride is distilled slowly through a short unpacked column until most of the acetic anhydride is removed. The residual solution is chilled, water is added, and the product is extracted with ether. The ether extract is washed with sodium hydroxide and water, dried over sodium sulfate, and the ether is removed. The dark residue is dissolved in petroleum ether and chromatographed on alumina. The colorless enol acetate recovered in the eluate is recrystallized from ethyl acetate, m.p. 200–201°; $[\alpha]_D^{33} + 105^\circ$ (chloroform).

(c) $3\alpha, 11, 20$ -Triacetox-17 $\alpha, 20$ -epoxy-9-pregnene-4,16,21- H^3 . The triacetate (b), 6.4 g., is dissolved in 30 ml. of 2.2 *M* perbenzoic acid in benzene, with intermittent cooling, and the solution is stored at room temperature for 2 hours. The solution is diluted with ether, extracted with base and then with water. Evaporation of the solvents leaves a crystalline product which is recrystallized from ethyl acetate, m.p. 195–196°; $[\alpha]_D^{25} + 77.0^\circ$ (chloroform).

(d) $3\alpha, 17\alpha$ -Dihydroxypregnane-11,20-dione-4,16,21- H^3 . The epoxide (Note 4) is saponified in 1 l. of 0.25 *N* sodium hydroxide in 50% ethanol at room temperature for 40 minutes. The product, a white crystalline solid, is recrystallized from benzene in plates, m.p. 198–201°. Recrystallization from ethyl acetate affords 3 crops of the dihydroxypregnandione: the major portion, m.p. 203–204°; a lesser fraction, m.p. 200–201°; and a small amount, m.p. 196–200° (Note 5).

(e) 21-Bromo- $3\alpha, 17\alpha$ -dihydroxypregnane-11,20-dione-4,16,21- H^3 . To a solution of 2.254 g. of $3\alpha, 17\alpha$ -dihydroxypregnane-11,20-dione-4,16,21- H^3 , dissolved in 45 ml. of chloroform (Note 6), is added 27.51 ml. of 0.247 *M* bromine in chloroform. This solution is diluted with 500 ml. of chloroform and washed with 5% sodium hydroxide and water. Removal of the chloroform *in vacuo* at low temperature leaves a white, crystalline product. After recrystallization from ethyl acetate to constant melting point, 2.25 g. of plates is obtained, m.p. 178–179.5°; $[\alpha]_D^{25} + 70^\circ$ (chloroform) (Note 7). The yield of bromo compound is 81%.

(f) $3\alpha, 17\alpha, 21$ -Trihydroxypregnane-11,20-dione-4,16,21- H^3 . A solution of 436 mg. of 21-bromo- $3\alpha, 17\alpha$ -dihydroxypregnane-11,20-dione-4,16,21- H^3 in 200 ml. of 95% ethanol is put under an atmosphere of nitrogen. At room temperature, 200 ml. of 0.1 *N* sodium hydroxide solution is added, and after 10 minutes the solution is acidified, diluted with ether, and washed with

small amounts of 5% solutions of sodium hydroxide and sodium chloride. After removal of the solvent, 270 mg. of the trihydroxypregnanedione, m.p. 191–192°, is obtained by crystallization of the residue from ethyl acetate-methanol. Recrystallization from ethyl acetate raises the m.p. to 195–196.5°; $[\alpha]_D^{31} + 69^\circ$ (acetone). The yield is 73%.

(g) 17 α ,21-Dihydroxypregnane-3,11,20-trione-4,16,21-H₆³. A solution of 107 mg. (0.294 mmole) of (f), above, in 3 ml. of *t*-butyl alcohol, containing 82.8 mg. (0.598 mmole) of N-bromoacetamide and 0.1 ml. of water, is stored for 5 hours. At that time, 0.297 mmole of the oxidizing agent has been consumed, and the solution is diluted with ethyl acetate and washed with 5% sodium hydroxide solution and water. The solvent is removed, leaving a white crystalline residue of 17 α ,21-dihydroxypregnane-3,11,20-trione-4,16,21-H₆³.

(h) 21-Acetoxy-17 α -hydroxypregnane-3,11,20-trione-4,16,21-H₆³. The dihydroxypregnanetrione (g) is acetylated with acetic anhydride and pyridine at room temperature to obtain 113 mg. of a yellow crystalline product. By chromatography followed by recrystallization from ethyl acetate, 25 mg. of 21-acetoxy-17 α -hydroxypregnane-3,11,20-trione-4,16,21-H₆³, m.p. 230–231°, and 60 mg. of m.p. 228–230° are obtained; the yield is 71%.

(i) 21-Acetoxy-4-bromo-17 α -hydroxypregnane-3,11,20-trione-4,16,21-H₆³. To a solution of 0.308 g. of 21-acetoxy-17 α -hydroxypregnane-3,11,20-trione-4,16,21-H₆³ in 10 ml. of acetic acid is added 0.3 ml. of 0.257 M bromine solution in acetic acid. The bromination is continued with 2.88 ml. of 0.251 M bromine in acetic acid buffered with an equimolar amount of sodium acetate. When the reaction is complete, ethyl acetate is added, and the solution is washed thoroughly with dilute sodium chloride solution, dilute sodium hydroxide, and again with the sodium chloride solution. The solvent is removed *in vacuo* at low temperature, and the residue is recrystallized from ethyl acetate, m.p. 196–199° (dec.); $[\alpha]_D^{27} + 103^\circ$ (chloroform) (Note 8).

(j) 21-Acetoxy-17 α -hydroxy-4-pregnene-3,11,20-trione-4,16,21-H₆³, (Cortisone-4,16,21-H₆³ Acetate). In an atmosphere of nitrogen, a solution containing 0.692 g. of 21-acetoxy-4-bromo-17 α -hydroxypregnane-3,11,20-trione-4,16,21-H₆³, 0.480 g. of semicarbazide hydrochloride and 0.480 g. of anhydrous sodium acetate is heated at 70° for 2 hours. Then, 7 ml. of pyruvic acid in 14 ml. of water is added, and the solution temperature is maintained at 70° for an additional 2 hours. The cooled solution is diluted with ethyl acetate and extracted thoroughly with dilute sodium hydroxide and with dilute sodium chloride solution. Evaporation of the solvent leaves 0.589 g. of cortisone-4,16,21-H₆³ acetate, which, after recrystallization from acetone, yields 0.469 g. (81%) of long plates, m.p. 245–246°; $[\alpha]_D^{29} + 186^\circ$ (acetone), + 218° (chloroform). The product is identical in all respects with a pure, authentic sample of cortisone acetate (Note 9).

(k) 17 α ,21-Dihydroxy-4-pregnene-3,11,20-trione-4,16,21-H₃³, (Cortisone-4,16,21-H₃³). A stream of nitrogen is bubbled through a solution of 2.00 g. of cortisone-4,16,21-H₃³ acetate in 1 l. of 95% alcohol for one-half hour. After the addition of 1 l. of 0.1 *N* sodium hydroxide solution, with stirring, the solution is kept for 40 minutes in a nitrogen atmosphere. Then the mixture is diluted with 10% sodium chloride and extracted with ethyl acetate. The extract solution is dried over sodium sulfate, and the solvent is removed *in vacuo*. The residue is cortisone-4,16,21-H₃³, m.p. 226–228° (evacuated capillary).

B. Notes

1. With the exception of the hydrogen exchange reaction, the synthesis of cortisone-4,16,21-H₃³ acetate is according to the procedure of Kritchevsky, Garmaise and Gallagher.²

2. Two more runs were made in the same manner, with reuse of the solvent each time.

3. The product is refluxed in the presence of alkali to remove all the isotope from the labile positions alpha to the carbonyl groups. It is presumed that during this treatment all, or very nearly all, of the isotope located at carbons 9,12,17 and 21 is replaced by ordinary hydrogen.

4. In the best preparative procedure, the intermediates need not be isolated and purified. The direct preparation from 3 α -acetoxypregnane-11,20-dione is given by Kritchevsky.²

5. The first crop is recrystallized without elevation of the melting point, $[\alpha]_D^{32} + 65.8^\circ$ (acetone).

6. Reagent grade chloroform is specified.

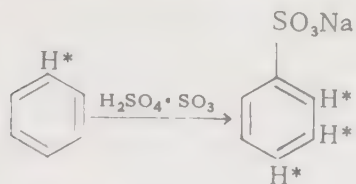
7. After five more recrystallizations, the melting point was still 178.5–179.5°. The residues were almost completely crystalline, and starting material could be obtained by debromination with zinc at room temperature.

8. Previous results indicated that it was advantageous to reduce residual crude material with zinc and again brominate it rather than to attempt purification by recrystallization.

9. Through degradation of the labeled 3 α ,17 α -dihydroxypregnane-11,20-dione, the stably bound tritium is deduced to be distributed as follows: 70% at C-16, 5% at C-21, 20% at C-4, and 5% not located.

¹C. W. Marshall, T. H. Kritchevsky, S. Lieberman and T. F. Gallagher, *J. Am. Chem. Soc.*, **70**, 1837 (1948).

²T. H. Kritchevsky, D. L. Gamaise and T. F. Gallagher, *ibid.* **74**, 483 (1952).

SODIUM BENZENESULFONATE-2,3,4- $H^3_{1/3}$ 

L. Melander, *Arkiv Kemi*, 2, 278 (1950).

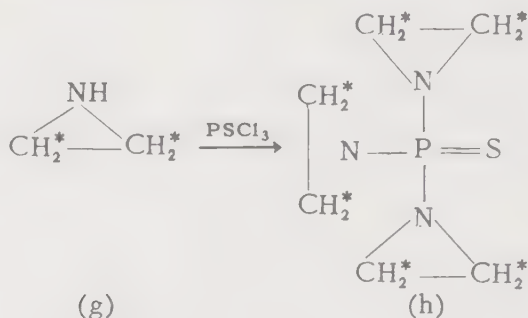
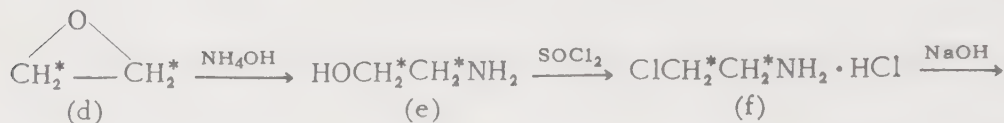
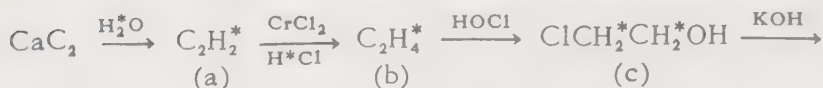
A. Procedure

To about 2 ml. of fuming sulfuric acid (10–12% SO_3) is added 1.00 ml. of benzene- H^3_1 . The flask is stoppered and cooled with water for about one-half minute; then the mixture is shaken for 8–15 minutes, with intermittent cooling at first. Finally, the reaction mixture is poured into 5 ml. of saturated sodium chloride solution, which is cooled in an ice-bath. The flask is rinsed with a few ml. of the same solution. After the salt solution is cooled in a refrigerator, the crystalline sodium benzenesulfonate-2,3,4- $H^3_{1/3}$ is collected and washed with saturated sodium chloride solution. The crystalline mass, partially dried on the filter, is boiled with three 60-ml. portions of alcohol. The combined extract is evaporated to dryness, and the residue is dissolved in absolute alcohol. The solution is filtered and evaporated until crystallization begins. Then the crystallization is completed in a refrigerator; the product is collected, partially dried on the filter, extracted twice with boiling ether and finally dried at 110° (Note 1). The yield is 53% (Note 2). The ratio of Y to Y_0 is 0.928, where Y is the ratio of isotopic to nonisotopic molecules in the product and Y_0 is the corresponding ratio in the starting material (Note 3).

B. Notes

1. The product contained very little sodium chloride.
2. Melander felt that the precipitation of product from the sodium chloride solution was incomplete.
3. The results indicated that tritium was substituted significantly more slowly than protium, in this instance. In a similar sulfonation reaction, starting with 1-bromobenzene-4- H^3 , which was interrupted before completion, the tritium content of the remaining 1-bromobenzene-4- H^3 (17%) had increased by 50%.

1,1',1''-PHOSPHINOTHIOLYDINETRISAZIRIDINE- H_{12}^3
(N,N',N'' -Trisethylenethiophosphoramide- H_{12}^3)



D. L. Williams, Los Alamos Scientific Laboratory, Los Alamos, New Mexico, unpublished work.

A. Procedure

(a) *Acetylene- H_2^3* . With a slow stream of dry nitrogen flowing through the all-glass apparatus, acetylene- H_2^3 is generated from 0.2 ml. of water- H_2^3 and an excess of calcium carbide and is collected in a U-trap cooled with liquid nitrogen. The flow of dry nitrogen is continued for 30 minutes (Note 1).

(b) *Ethylene- H_4^3* . The U-trap is connected to a vacuum manifold, and the acetylene- H_2^3 is distilled *in vacuo* into a 200-ml. pressure flask containing chromous chloride solution (Note 2). The flask is removed from the vacuum line and shaken mechanically for 12.5 hours. After the flask is reattached to the vacuum line, it is cooled in liquid nitrogen and again evacuated. Ethylene- H_4^3 is then distilled from the reaction mixture at a bath temperature of -78° . When most of the ethylene- H_4^3 has been collected, the reaction mixture is melted and refrozen in liquid nitrogen, and the distillation is continued at -78° (Note 3).

(c) *2-Chloroethanol-1,2- H_4^3* . Into a 100-ml. flask equipped with a 2-mm. pressure stopcock is transferred 5.0 ml. of 4.4 M hypochlorous acid solution (Note 4) and 5.0 ml. of water. The ethylene- H_4^3 is then distilled into the flask *in vacuo*. The flask is detached from the vacuum manifold and shaken mechanically for 2 hours.

(d) *Ethylene- H_4^3 Oxide*. The above flask containing 2-chloroethanol-1,2- H_4^3 and excess hypochlorous acid is attached to the vacuum manifold

and cooled with liquid nitrogen. After the flask is evacuated, chlorine is distilled from the aqueous mixture at -78° . Then the entire aqueous solution is distilled into a 200-ml. flask containing 6 g. of powdered potassium hydroxide and a magnetic stirring bar. As the aqueous solution melts, a rapid evolution of ethylene- H_4^3 oxide occurs. The volatile material is condensed on the potassium hydroxide, which is stirred magnetically, several times to ensure complete reaction.

(e) *2-Aminoethanol-1,2- H_4^3* . The ethylene- H_4^3 oxide is distilled *in vacuo* into a 200-ml. flask containing 17 ml. of frozen concentrated ammonium hydroxide (Note 5) and a magnetic stirring bar. The solution is stirred for several hours at room temperature and is then heated in a bath at 55° for 7 hours. The flask is cooled, removed from the vacuum manifold and fitted with a reflux condenser. After 10 ml. of water is added, 35 g. of anhydrous potassium carbonate is added slowly with stirring during several hours (Note 6). When all the carbonate has dissolved, the solution is transferred to a continuous-type extractor (Note 7), and the condenser, flask and funnel are washed with a total of 25 ml. of water. Finally, 10 g. more solid potassium carbonate is dissolved in the solution (Note 8). The resulting solution is extracted continuously with chloroform for a total of 64 hours. The extract is removed at intervals and filtered into a flask containing excess concentrated hydrochloric acid. The total yield of 2-aminoethanol-1,2- H_4^3 hydrochloride, upon evaporation of the mixture to dryness, is 0.2640 g. (2.71 mmoles).

(f) *2-Chloroethylamine-1,2- H_4^3 Hydrochloride*. To the above 2.71 mmoles of amine salt is added 8.35 mmoles of 2-aminoethanol hydrochloride and a solution of 5 ml. of purified thionyl chloride in 20 ml. of 1,1,1-trichloroethane. The mixture, protected from moisture by a calcium chloride tube, is refluxed for 8 hours (Note 9). Then the mixture is treated under reflux with methanol in slight excess to destroy residual thionyl chloride (Note 10). After the addition of 10 ml. of 1,1,1-trichloroethane, the volume of solvent is reduced to 5-10 ml. by distillation. The mixture is cooled, and the residual solvent is removed from the nearly colorless crystalline product (Note 11), which weighs 1.2165 g. (10.5 mmoles).

(g) *Aziridine-2,3- H_4^3 , (H_4^3 -Ethylenimine)*. The above 2-chloroethylamine-1,2- H_4^3 hydrochloride is dissolved in methanol and transferred into a 25-ml. reaction flask equipped with a 4-inch sealed-on water condenser and a semiball joint. The solvent is distilled off *in vacuo*, first at -20° and then at room temperature. Then the apparatus is detached from the manifold and cooled in a bath at -10° while 7.9 ml. of 4 *N* sodium hydroxide solution is added (Note 12). The flask is reattached to the vacuum manifold and, with water at 10° circulating in the condenser, the mixture is heated for 1 hour in a water-bath at 50° . Follow-

ing the heating period, the circulating water is shut off, and the water is completely removed from the condenser with a stream of air. The reaction flask, including the condenser, is then immersed in liquid nitrogen. The reaction flask, containing the imine, and a 100-ml. flask, which contains 40 g. of solid sodium hydroxide and is also cooled in liquid nitrogen, are evacuated. The imine and most of the water in the reaction mixture are distilled *in vacuo* into the flask containing the solid sodium hydroxide. As the water melts it distills onto the cold mass of sodium hydroxide. When the entire mixture is at room temperature, the aziridine-2,3- H_4^3 and a small amount of water are distilled *in vacuo* into a flask containing 5 g. of freshly ignited calcium oxide. After 26 hours, the dry imine is distilled into a tared, evacuated weighing flask. The yield of aziridine- H_4^3 is 0.2655 g. (61.7%) (Note 13).

(h) 1, 1', 1''-Phosphinothioylidynetrisaziridine- H_{12}^3 , (N,N',N''-Trisethylenethiophosphoramidate- H_{12}^3). The above aziridine- H_4^3 is distilled *in vacuo* into a 100-ml. 2-necked flask containing 20 ml. of benzene, 1.24 ml. (24.06 mmoles) of ethylenimine and 4.2 ml. (30 mmoles) of triethylamine. With the reagents still frozen, the flask is filled with dry nitrogen and is then warmed until the benzene is melted. The flask is removed from the vacuum manifold and quickly fitted with a mercury-sealed stirrer and a compensated dropping funnel. To the stirred reaction mixture at 0–3° is added dropwise a solution of 1.038 ml. (1.6945 g., 10 mmoles) of thiophosphoryl chloride in 10 ml. of benzene (Note 14). Then 0.2 ml. of excess ethylenimine is added (Note 15), and stirring is continued for 45 minutes as the solution is let warm to room temperature. The reaction mixture is diluted with about 30 ml. of ether and is then filtered through a medium sintered glass funnel. The precipitate of triethylamine hydrochloride is washed several times with ether. The combined filtrate is evaporated under low vacuum. The residue (Note 16) is extracted repeatedly with ether, and the extracts are filtered and combined. Evaporation of the ether leaves a colorless, crystalline crude product, 1.8953 g. The crude product is dissolved in 65 ml. of warm petroleum ether (Note 17); the solution is filtered and cooled to –40 to –50° for 2 hours. Removal of the solvent with a filter stick leaves 1.5918 g. (84.2%) of crystalline product. The product is dissolved in 45 ml. of warm hexane (water-bath at 60°), which is filtered and cooled at 0° overnight and for an additional 2 hours at –40°. The solvent is removed with a filter stick, and the crystalline product is dried to constant weight under low vacuum. The yield of pure product (Note 18), m.p. 53–54°, is 1.5350 g. (81.0%, based on thiophosphoryl chloride).

B. Notes

1. This was to ensure transfer of all the water- H_2^3 from the glass to the carbide and collection of all the product in the trap.

2. The chromous chloride solution was prepared from 12 g. of chromic chloride hexahydrate, 23.5 ml. of water- H_2^3 and 5.5 ml. of concentrated hydrochloric acid by reduction with amalgamated mossy zinc.¹ The concentration of tritium in the water was adjusted such that the average concentration of tritium in the acidic medium was equal to that in the acetylene- H_2^3 . This preparation of ethylene- H_4^3 from acetylene- H_2^3 is similar to the procedure for ethylene- C_2^{14} described by Cox and Warne.²

3. Yields of ethylene in preliminary runs were 99–100%; the yield in this instance, based on water- H_2^3 used to prepare acetylene- H_2^3 , was approximately 40%.

4. A convenient preparation of pure, aqueous hypochlorous acid solution from mercuric oxide and chlorine is described by Reformatzky.³ The hypochlorous acid solution was analyzed⁴ by electrometric titration with sodium arsenite.

5. According to Pilgeram, *et al.*,⁵ in order to realize a high yield of 2-aminoethanol (80–90%), it is imperative to use at least a 50-mole excess of ammonium hydroxide.

6. The evolution of ammonia from the solution is rapid and the carbonate is introduced through the condenser by means of a long-stemmed funnel to avoid loss of product.

7. An ether-type extractor is used.

8. 2-Aminoethanol-1,2- H_4^3 appears in droplets on the surface of the aqueous solution, which now has a specific gravity greater than that of chloroform.

9. The amine hydrochloride appears to melt and float on the surface of the reagent mixture in syrupy globules. As the reaction progresses, 2-chloroethylamine hydrochloride crystallizes, and the melt becomes entirely solid.

10. In preliminary experiments, the product was found to codistill with a mixture of thionyl chloride and 1,1,1-trichloroethane.

11. Most of the colored by-products are soluble in the solvent. In a preliminary experiment starting with pure 2-aminoethanol hydrochloride, the yield of crude 2-chloroethylamine hydrochloride, m.p. 140–142°, was 98.5%. Yields of crude product of 97% and 99% have been reported when the reaction was run in toluene⁶ and chloroform,⁷ respectively. M.p. of the product recrystallized from ethanol was 148.5–150°.⁶

12. This procedure is an adaptation of that described by Wystrach, *et al.*⁶ The molar ratio of sodium hydroxide to 2-chloroethylamine hydrochloride is 3 to 1.

13. In preliminary runs, the yields of ethylenimine from pure 2-chloroethylamine hydrochloride ranged from 78 to 89%. In a similar preparation of aziridine- C_2^{14} , the yield was 83.4%. In some instances part (<10%) of the ethylenimine polymerized in the weighing flask on standing several hours.

14. This procedure is an adaptation of that outlined by Kuh, *et al.*⁸

15. Since each mole of thiophosphoryl chloride reacts with 3 moles of the imine, excess imine was added to ensure replacement of all 3 of the chlorine atoms after the labeled imine was consumed.

16. The residue contains a considerable amount of triethylamine hydrochloride.

17. A residue of viscous, noncrystalline material is left.

18. The radioautograph of a paper strip chromatogram indicated only one labeled compound in the product.

¹M. R. Hatfield, *Inorganic Syntheses*, Vol. III, McGraw-Hill, New York, 1950, p. 148.

²J. D. Cox and R. J. Warne, *J. Chem. Soc.*, 1951, 1893.

³S. Reformatzky, *J. prakt. Chem.*, 40, 396 (1889).

⁴E. Müller and H. Dietmann, *Z. Anal. Chem.*, 73, 138 (1928).

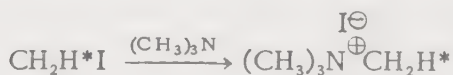
⁵L. O. Pilgeram, E. M. Gal, E. N. Sassenrath and D. M. Greenberg, *J. Biol. Chem.*, 204, 367 (1953).

⁶V. P. Wystrach, D. W. Kaiser and F. S. Schaefer, *J. Am. Chem. Soc.*, 77, 5915 (1955).

⁷G. W. Raiziss and L. W. Clemence, *ibid.*, 63, 3124 (1941).

⁸E. Kuh, N. Brunswick and D. R. Seeger, U. S. 2,670,347, Feb. 23, 1954.

TETRAMETHYLAMMONIUM-H₁³ IODIDE



D. Harman, T. D. Stewart and S. Ruben, *J. Am. Chem. Soc.*, 64, 2294 (1942).

A. Procedure (Note 1)

To 10 ml. of 0.2 M trimethylamine solution in a 25-ml. flask is added 5 ml. of 0.2 M methyl-H₁³ iodide solution. After shaking, the mixture is placed in a bath at 25° (Note 2). After about 3 hours, the excess of amine and the solvent are removed from the salt by distillation under vacuum (Note 3). The contents of the flask, which is connected to a second flask with wide-bore tubing, are frozen in liquid air, the system is evacuated, the liquid air-bath is placed around the second flask, and the reaction mixture is gently warmed (Note 4). The residue in the first flask is the quaternary salt, tetramethylammonium-H₁³ iodide.

B. Notes

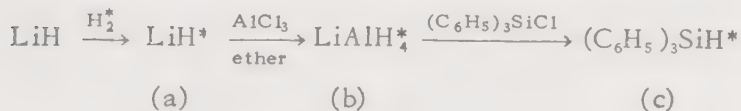
1. The following procedure was used for both benzene and alcohol solutions.

2. When the reaction is carried out in alcohol, crystals begin to form in about 10 minutes; with benzene as solvent an intermediate turbidity is observed.

3. The reaction reaches completion in 3 hours.

4. By radioactivity measurements on hydrogen prepared from the unreacted amine it was shown that none of the tritium appears in the unreacted amine through an exchange of methyl groups by an equilibrium process.

TRIPHENYLSILANE-H³



L. Kaplan and K. E. Wilzbach, J. Am. Chem. Soc., 74, 6152 (1952).

A. Procedure

(a) *Lithium Hydride-H³*. Lithium hydride-H³ is prepared by an exchange process. According to the procedure of Wilzbach and Kaplan,¹ 200 mesh lithium hydride is heated with hydrogen-H₂³ in a Pyrex flask at 350° (Note 1).

(b) *Lithium Aluminum Hydride-H³*. This compound is prepared according to the procedure of Finholt, Bond and Schlesinger² for the preparation of nonisotopic lithium aluminum hydride. In a typical example, an excess of lithium hydride, 23.5 g. (2.96 moles) (Note 2), is added to a solution of 3.05 g. (0.08 mole) of lithium aluminum hydride in 30 ml. of ether (Note 3), and the mixture is stirred (Note 4) for a short time. After the addition of 200 ml. more ether, a solution of 71.2 g. (0.534 mole) of aluminum chloride in 300 ml. of ether is introduced at such a rate that boiling of ether is continuous. The mixture is stirred during the addition and for a short time after the reaction appears to have ceased. The precipitated lithium chloride and the excess of lithium hydride are separated from the solution of lithium aluminum hydride by filtration under nitrogen pressure.

This procedure gives yields of about 86% of a product that is 95.4% lithium aluminum hydride. Higher yields are obtained if the solution stands a longer time before filtration. The purity of the product also improves; it may become as high as 99% without recrystallization.

(c) *Triphenylsilane-H³*. This compound is prepared according to the general method of Gilman and Dunn³ for the preparation of triarylsilanes. To a solution of 0.055 mole of chlorotriphenylsilane dissolved in 125 ml. of dry ether is added 1 g. (0.026 mole) of lithium aluminum hydride-H₄³. The mixture, protected from atmospheric moisture, is heated under reflux for 3 hours. The excess lithium aluminum hydride-H₄³ is hydrolyzed by adding ether saturated with water, followed by dilute aqueous acetic acid. The

ether layer is washed with dilute acetic acid, dried over sodium sulfate and distilled at reduced pressure after the removal of solvent.

B. Notes

1. Exchange is observed at room temperature and is substantially complete at 200° within 24 hours.

2. Unless the lithium hydride is finer than 100 mesh, it reacts with aluminum chloride very slowly.

3. The addition of a small amount of previously prepared lithium aluminum hydride prevents a troublesome phenomenon. Otherwise there is an induction period that may last several minutes or several hours, followed by reaction so vigorous that it may not be controlled by cooling the mixture.

4. The reaction is carried out in a three-necked flask fitted with a mercury-sealed stirrer, a reflux condenser, and a dropping funnel. Use of a dry, carbon dioxide-free atmosphere during the reaction and filtrations is advantageous.

C. Other Preparations

Preparation of a small quantity of lithium aluminum hydride, using a vacuum system, has also been described by Kaplan and Wilzbach. This procedure does not include the addition of lithium aluminum hydride to initiate the reaction and might be employed profitably in the synthesis of lithium aluminum hydride- H_4^3 .

An attempt to prepare lithium aluminum hydride- H_4^3 by the direct exchange of commercial lithium aluminum hydride with hydrogen- H_2^3 at 100° was unsuccessful.

Kaplan and Wilzbach⁴ have also prepared triphenylsilane- H^3 and tripropylsilane- H^3 by reduction of the corresponding chlorosilane similarly to the procedure described. These compounds were used in a study of hydrogen isotope effects in the alkaline cleavage of triorganosilanes.

¹K. E. Wilzbach and L. Kaplan, J. Am. Chem. Soc., 72, 5795 (1950).

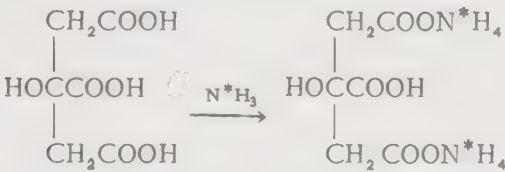
²A. E. Finholt, A. C. Bond, Jr., and H. I. Schlesinger, *ibid.*, 69, 1199 (1947).

³H. Gilman and G. E. Dunn, *ibid.* 73, 3404 (1951).

⁴L. Kaplan and K. E. Wilzbach, *ibid.*, 77, 1297 (1955).

NITROGEN-15 COMPOUNDS

BIS(AMMONIUM-N¹⁵) CITRATE



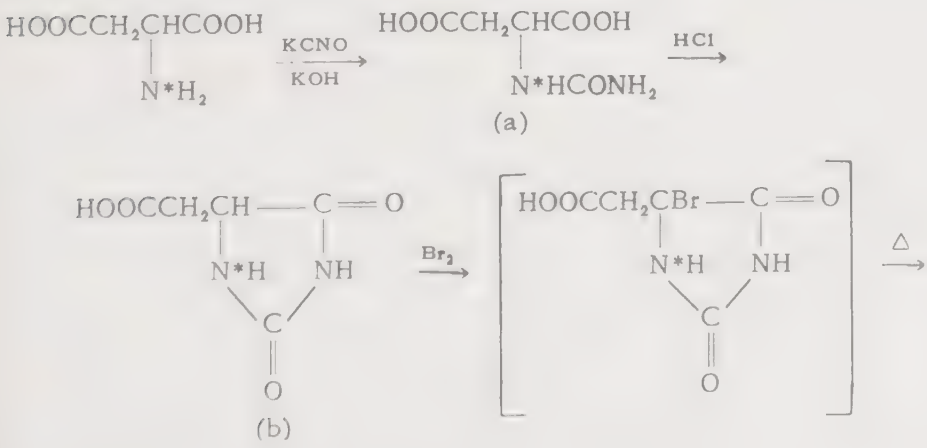
H. D. Hoberman and J. Graff, J. Biol. Chem., 186, 373 (1950).

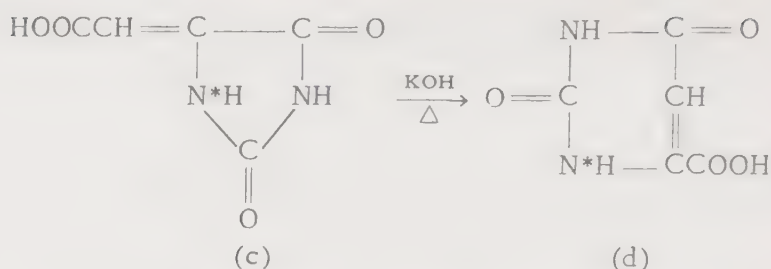
Procedure

Bisammonium-N¹⁵ citrate is prepared as an aqueous solution by distilling ammonia-N¹⁵ into the calculated amount of citric acid dissolved in water.

OROTIC-3-N¹⁵ ACID

(1,2,3,6-Tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic-3-N¹⁵ Acid)





H. Arvidson, N. A. Eliasson, E. Hammersten, P. Reichard, H. v. Ubisch and S. Bergström, J. Biol. Chem., 179, 169 (1949).

A. Procedure

Although Arvidson and co-workers prepared the isotopic orotic acid, no experimental details were given. Therefore, the following procedure is taken from the original work of Nyc and Mitchell.¹

(a) *Ureidosuccinic-1-N¹⁵ Acid*. A mixture of 13 g. of aspartic acid, 8 g. of potassium cyanate, and 100 ml. of 1 N potassium hydroxide is set aside at room temperature for 16 hours. The solution is acidified with concentrated hydrochloric acid and after two hours the product is collected and recrystallized from water. The yield is 10.5 g. (68%), m.p. 178–180°.

(b) *5-Hydantoinacetic-1-N¹⁵ Acid*. The ureidosuccinic acid, 10.5 g., is dissolved in 25 ml. of 20% hydrochloric acid, and the mixture is evaporated nearly to dryness on a hot plate. The residue is recrystallized from water; the yield of the hydantoin is 7.9 g. (84%), m.p. 214–216° (Note 1).

(c) *5-Carboxymethylenehydantoin-1-N¹⁵*. A mixture of 1.28 g. of bromine, 4 g. of 5-hydantoinacetic acid and 16 ml. of glacial acetic acid, in a sealed tube, is heated at 100° for 1.5 hours, with shaking. Upon cooling the mixture, the precipitated product is collected and suspended in boiling water for 20 minutes. Water is then added to the boiling suspension until all of the solid has dissolved. When the solution is cooled, 5-carboxymethylenehydantoin crystallizes; the yield is 2.92 g. (73%), m.p. above 400°.

(d) *Orotic-3-N¹⁵ Acid*, (1,2,3,6-Tetrahydro-2,6-dioxo-4-pyrimidinecarboxylic-3-N¹⁵ Acid). 5-Carboxymethylenehydantoin, 0.60 g., is dissolved in 20 ml. of 1 N potassium hydroxide in a constant temperature bath at 64°³ (Note 2). After 2 hours, the solution is acidified with concentrated hydrochloric acid, and the precipitated orotic acid is collected and recrystallized from water. The yield of anhydrous compound is 0.557 g. (93%); decomposition temperature of sample immersed at 320° is 343–345°.

B. Notes

1. Gabriel² obtained this compound directly from aspartic acid without isolation of ureidosuccinic acid, (a); however, Nyc and Mitchell found it

advantageous to isolate and purify the latter since it is the less soluble of the two and more easily purified.

2. The course of the reaction was followed by absorption spectra determinations on samples taken at 15-minute intervals and diluted with 0.1 M potassium hydroxide solution. The curves are given in the original literature.

C. Other Preparations

Reichard and Lagerkvist⁴ also prepared L-ureidosuccinic-1-N¹⁵ acid, by the method of Nyc and Mitchell,¹ from 0.8 g. of L-aspartic-N¹⁵ acid. They found that L-ureidosuccinic acid, unlike the racemic form, did not crystallize from the acidified reaction mixture. Therefore, an isolation procedure was developed which involved chromatographic separation on Dowex-2 (Cl-form) ion exchange resin. The product was finally isolated as barium L-ureidosuccinate-1-N¹⁵, which did not melt below 300°; $[\alpha]_D^{25} +24.1^\circ$ (c, 3% in water).

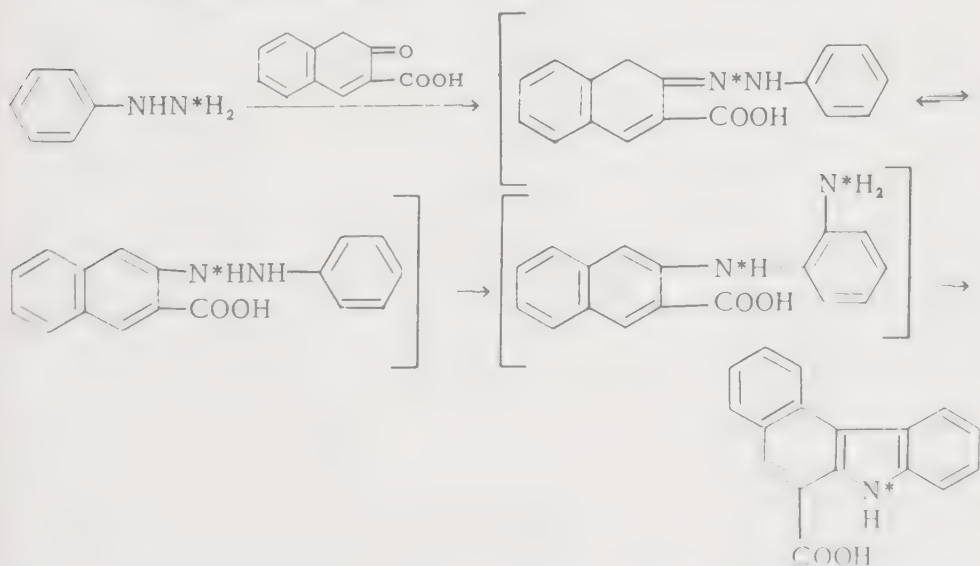
¹J. F. Nyc and H. K. Mitchell, J. Am. Chem. Soc., 69, 1382 (1947).

²S. Gabriel, Ann., 348, 50 (1906).

³H. K. Mitchell and J. F. Nyc, J. Am. Chem. Soc., 69, 674 (1947).

⁴P. Reichard and U. Lagerkvist, Acta Chem. Scand., 7, 1207 (1953).

7H-BENZO[c]CARBAZOLE-6-CARBOXYLIC-N¹⁵ ACID



K. Clusius and M. Barsh, Helv. Chim. Acta, 37, 2013 (1954).

A. Procedure

In a reaction flask which can be attached to vacuum apparatus, 0.385 g. (3.56 mmoles) of 1-phenylhydrazine-2-N¹⁵ is added to 0.644 g. (3.42

mmoles) of 3-hydroxy-2-naphthoic acid. After the mixture is frozen in liquid nitrogen, the flask is evacuated and then filled to atmospheric pressure with hydrogen which is purified by passage through a trap cooled in liquid hydrogen. The mixture is heated and melts between 130–140° to a homogeneous amber-colored liquid. Then, gas evolution begins, and at 150° crystals appear and the mixture solidifies. While the mass is cooling, the apparatus is slowly evacuated, and all the gaseous products are distilled into a trap (Note 1). The brownish mass remaining in the flask is finely pulverized and extracted several times with hot alcohol. The yield of 7*H*-benzo[*c*]carbazole-6-carboxylic-N¹⁵ acid is 0.12 g. (about 14%). The product is dissolved in dilute potassium hydroxide solution and reprecipitated by the addition of dilute acetic acid. It is then recrystallized from acetic acid; the yield is 0.075 to 0.095 g. of material which sinters at 325° and rapidly turns black (Note 2).

B. Notes

1. The volatile by-products: a trace of aniline, unused phenylhydrazine, ammonia and nitrogen were separated, and the latter two were analyzed for nitrogen-15. The N¹⁵-content of the free nitrogen gas indicated that a simple cracking of the 1-phenylhydrazine-2-N¹⁵ had not occurred since it was equal to 82% of the excess N¹⁵ in the terminal amino group.

2. Analysis of the final product for N¹⁵ indicated an amount equal to all that present in the -NH- group of the original 1-phenylhydrazine-2-N¹⁵ plus 12% of the N¹⁵ in the -N¹⁵H₂ group.

CYANAMIDE-N₁¹⁵



A. Bendich, J. F. Tinker and G. B. Brown, *J. Am. Chem. Soc.*, 70, 3109 (1948).

A. Procedure

Ammonia-N¹⁵ is liberated by heating 9 g. of ammonium-N¹⁵ nitrate for 2 hours under reflux with 15 ml. of 12 *N* potassium hydroxide. The ammonia is aerated through a sodium hydroxide drying tower into a tube containing 65 ml. of anhydrous ethanol at -70°. Freshly distilled cyanogen bromide, 6.0 g. in 45 ml. of dry ether, is added, and the tube is securely stoppered and kept at room temperature for 16 hours. The isotopic ammonium bromide that has formed is collected, and the filtrate is evaporated to dryness *in vacuo* below 40°. The cyanamide-N₁¹⁵ is taken up in absolute ether, and the undissolved ammonium-N¹⁵ bromide is combined with the first crop (Note 1). The ether solution is concentrated

to dryness, and 2.18 g. (93%) of cyanamide- $N_{1/2}^{15}$ is obtained (Note 2). This is essentially the procedure of Bloch, *et al.*,¹ with modifications.

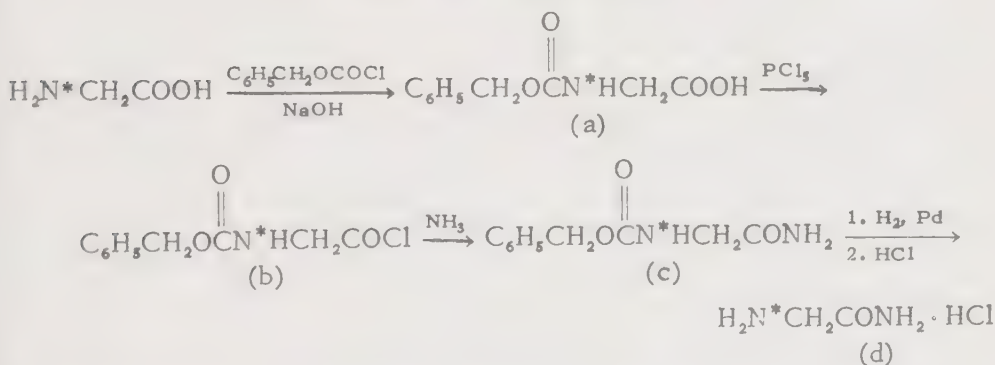
B. Notes

1. Total recovery was 5.05 g. (93%); in other experiments total recovery was 97, 100 and 98%.

2. The crystalline product is stored in a desiccator in the refrigerator.

¹K. Bloch, R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, **138**, 161 (1941).

2-AMINOACETAMIDE-2- N^{15}



H. D. Hoberman and D. Stone, *J. Biol. Chem.*, **194**, 383 (1952).

A. Procedure

(a) *N*-Benzyloxycarbonylglycine- N^{15} . According to the following procedure of Bergmann,¹ a solution of 7.5 g. of glycine in 25 ml. of 4 *N* sodium hydroxide is treated during 20 minutes, with ice-cooling and constant shaking, with a total of 17 g. of benzyloxycarbonyl chloride (Note 1) and 25 ml. of 4 *N* sodium hydroxide added in 5 portions. Upon acidification of the solution, crystals soon form. After recrystallization from chloroform, the yield of product, m.p. 120° (cor.), is 15 g. (72%) (Note 2).

(b) *N*-Benzyloxycarbonylglycyl- N^{15} Chloride. In the procedure of Bergmann,¹ a mixture of 6.3 g. of *N*-benzyloxycarbonylglycine, 35 ml. of dry ether and 6.7 g. of powdered phosphorus pentachloride is shaken for 20 minutes with ice-cooling. Most of the solid dissolves, and the solution is filtered and evaporated under reduced pressure, with the exclusion of moisture. The residue is thoroughly shaken with three portions of dry petroleum ether, which are combined and cooled to -10° until the product crystallizes. The product, 5.3 g., is recrystallized from ether-petroleum ether and then melts at 43° (Note 3).

(c) 2-(Benzyloxycarbonylamino)acetamide-2- N^{15} . Into a solution of 4 g. of N -benzyloxycarbonylglycyl- N^{15} chloride in dry ether is passed dry ammonia in excess, with cooling. The yield of amide is 2.1 g., m.p. 145° .

(d) 2-Aminoacetamide-2- N^{15} Hydrochloride. A solution of 2.1 g. of 2-(benzyloxycarbonylamino)acetamide-2- N^{15} in a mixed solvent of aqueous methanol and acetic acid is hydrogenated with a catalyst of palladium-black in an open vessel (Note 4). After about 30 minutes the evolution of carbon dioxide ceases. The solution is filtered, hydrochloric acid is added, and the filtrate is evaporated under reduced pressure. The yield of 2-aminoacetamide-2- N^{15} hydrochloride, m.p. $190-193^{\circ}$, is 0.68 g.

B. Notes

1. The preparation of benzyloxycarbonyl chloride from phosgene and benzyl alcohol is described.¹

2. This compound is easily soluble in alcohol and slightly soluble in water and most organic solvents.

3. Upon melting, N -benzyloxycarbonylglycyl chloride decomposes to form N -carboxyglycine anhydride and benzyl chloride.

4. The catalytic hydrogenation of benzyl-oxygen and -nitrogen bonds has been studied by Rosenmund² and others.^{3,4}

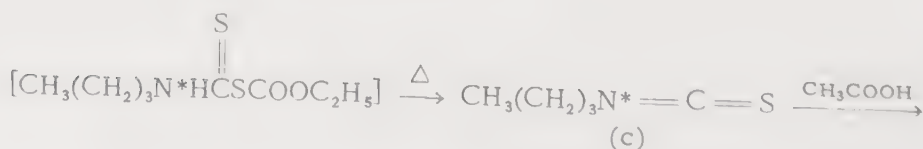
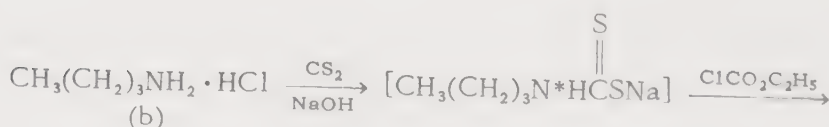
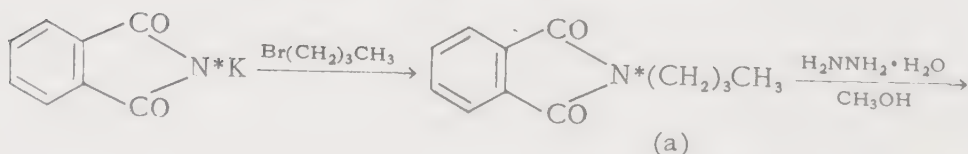
¹M. Bergmann and L. Zervas, *Ber.*, 65, 1192 (1932).

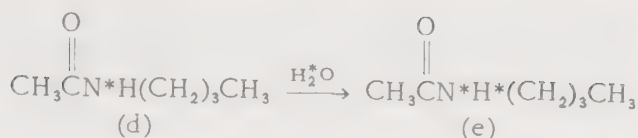
²K. W. Rosenmund and F. Zetzsche, *ibid.*, 54, 2038 (1921).

³K. Freudenberg, W. Dürr and H. v. Hochstetter, *ibid.*, 61, 1735 (1928).

⁴H. O. L. Fischer and E. Baer, *ibid.*, 65, 337, 345 (1932).

N -BUTYLACETAMIDE- N - H^2 - N^{15}





W. R. Vaughan, M. V. Andersen, Jr., H. S. Blanchard, D. I. McCane and W. L. Meyer, *J. Org. Chem.*, **20**, 819 (1955).

A. Procedure

(a) *N*-Butylphthalimide- N^{15} . In a 100-ml. flask equipped with a stirrer and condenser, 7.4 g. (5.7 ml., 0.054 mole) of butyl bromide is added to a mixture of 10 g. (0.054 mole) of potassium phthalimide- N^{15} and 25 ml. of *N,N*-dimethylformamide.¹ The resulting mixture is heated at 70°, with stirring, for 4 hours (Note 1). At the end of the heating period, the mixture is cooled to 0° and filtered (Note 2). After removal of dimethylformamide under partial vacuum, the yield of crude *N*-butylphthalimide- N^{15} is 12.52 g. (Note 3).

(b) *Butylamine- N^{15} Hydrochloride*. To the above crude imide is added 2.7 g. (0.054 mole) of hydrazine hydrate; the resulting mixture is heated on a steam-bath for 1 hour, and during this time a white solid separates. After 20 ml. of water is added, the methanol is removed under partial vacuum. Then, 20 ml. of concentrated hydrochloric acid is added, and the mixture is heated for 1 hour on a steam-bath. The mixture is cooled, and 8.6 g. (98.3%) of phthalhydrazide is removed by filtration. The filtrate is warmed on a hot plate and evaporated to dryness with an airstream. The yield of crude butylamine- N^{15} hydrochloride is 7.9 g.

(c) *Butyl Isothiocyanate- N^{15}* (Note 4). The crude butylamine- N^{15} hydrochloride is dissolved in water in a flask equipped with an efficient stirrer and condenser. To the stirred solution is added 4.1 g. (0.054 mole) of reagent carbon disulfide, and 40% sodium hydroxide solution is added dropwise until the pH is adjusted to 10–11. Then, 2.16 g. (0.054 mole) of sodium hydroxide in 3 ml. of water is added dropwise during 5–10 minutes. The resulting mixture is heated gently on a steam-bath until an oil separates, after which the mixture is cooled, and 5.9 g. (5.4 ml., 0.054 mole) of ethyl chlorocarbonate (Note 5) is added over a period of 5–10 minutes. The mixture is warmed on a steam-bath until the visible evolution of carbon oxysulfide ceases. The yield of crude butyl isothiocyanate- N^{15} is 6.0 g. (97%); b.p. 141° (30 mm.) (cor.), n_D^{25} 1.4404 after fractionation.

(d) *N*-Butylacetamide- N^{15} . A solution of the crude butyl isothiocyanate in 5 ml. of acetic acid is heated in an oil-bath maintained at 160°. After about 9 hours, the color of the solution becomes dark brown and the temperature reaches 155°; heating is continued for 3 more hours. Frac-

tionation of the reaction mixture yields 3.75 g. (67% based on crude isothiocyanate) of *N*-butylacetamide- N^{15} , b.p. 144.5–146.0° (33 mm.).

(e) *N*-Butylacetamide- $N-H^2-N^{15}$. A 1.0-ml. amount of *N*-butylacetamide- N^{15} is mixed with 4.0 ml. of 99–100% water- H^2 , giving a somewhat cloudy solution. The mixture is prepared in a micro-distillation flask, which is then connected to a total reflux distillation column and placed in an oil-bath at 150–160°. The water is removed by distillation (column temperature 110°), and the residue in the flask is heated at 150° for an additional two hours. The remaining oil is dried over phosphorus pentoxide in a vacuum desiccator for 12 hours (Note 6).

B. Notes

1. During this time potassium bromide separates, and the mixture becomes light tan in color.

2. The potassium bromide removed was 6.25 g. or 97.5% of the theoretical amount.

3. It was found advantageous to use each reaction product, for the next step in the series, without isolation or purification. Under ideal conditions, an over-all 93% yield of butyl isothiocyanate was possible.

4. The procedure is adapted from that used for the preparation of methyl isothiocyanate.²

5. The reaction of ethyl chlorocarbonate with the dithiocarbamate affords a higher yield of the isothiocyanate than steam distillation of the lead salt.³

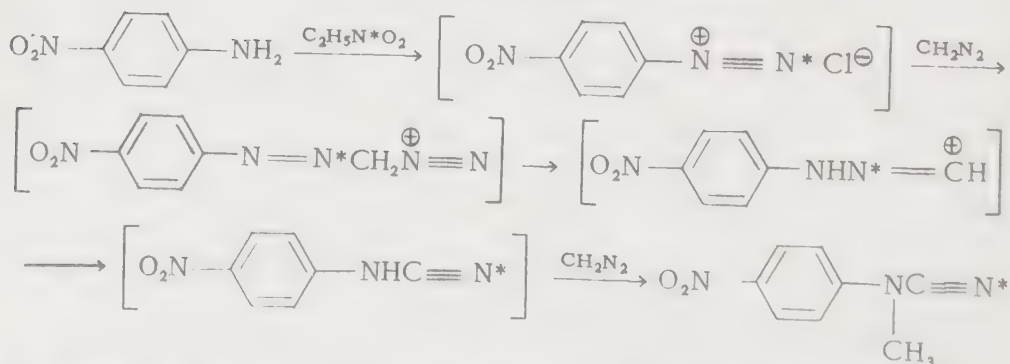
6. This sample showed practically no absorption due to the N-H bond.

¹J. C. Sheehan and W. A. Bolhofer, J. Am. Chem. Soc., 72, 2786 (1950).

²Organic Syntheses, Coll. Vol. III, Wiley, New York, 1955, p. 599.

³D. E. Worrall, J. Am. Chem. Soc., 50, 1456 (1928).

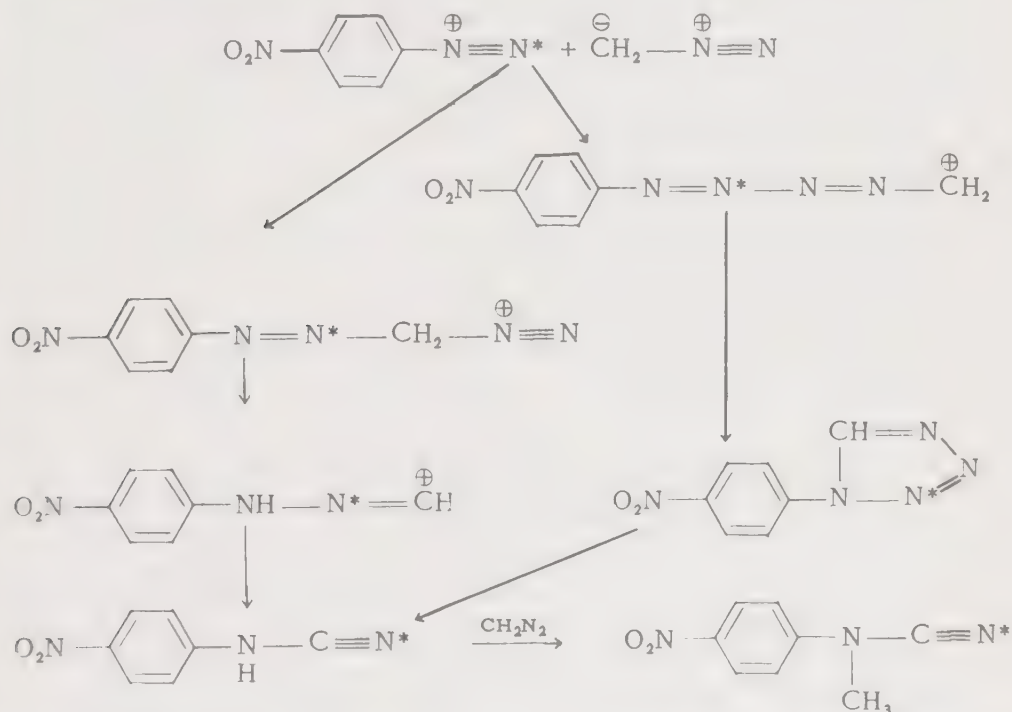
N-METHYL-4-NITROCARBANILONITRILE- N^{15} (Methyl-4-nitrophenylcyan- N^{15} -amide)



K. Clusius, H. Hürzeler, R. Huisgen and H. J. Koch, Naturwissenschaften, 41, 213 (1954); K. Clusius, Angew. Chem., 66, 497 (1954).

A. Procedure

4-Nitroaniline is diazotized with ethyl nitrite- N^{15} to obtain the solid 4-nitrobenzenediazonium- β - N^{15} salt which is coupled with diazomethane, in excess, according to the procedure of Huisgen and Koch¹ (Note 1). In addition to a 24% yield of methyl-4-nitrophenylcyan- N^{15} -amide, up to 8% of 1-(4-nitrophenyl)tetrazole-2- N^{15} was found (Note 2). Two possible mechanisms, presented below, for the coupling reaction are then possible, and the question arises whether or not the tetrazole derivative is an intermediate in the formation of 4-nitrophenylcyan- N^{15} -amide and methyl-4-nitrophenylcyan- N^{15} -amide.



When the final product is hydrolyzed with acid, 98% of the nitrite nitrogen appears as ammonia- N^{15} with an isotope content equivalent to that in the original nitrite. Thus, the carbonium ion rearrangement mechanism is responsible for the formation of all the 4-nitrophenylcyanamide, under the conditions employed.

B. Notes

1. Chloroformal *p*-nitrophenylhydrazone was isolated in 85% yield when a solution of diazomethane was dropped slowly into a cold, stirred methanolic solution of *p*-nitrobenzenediazonium chloride saturated with lithium chloride. Whether tautomerization from azo to hydrazone configuration took place before or after the capture of a chloride anion was

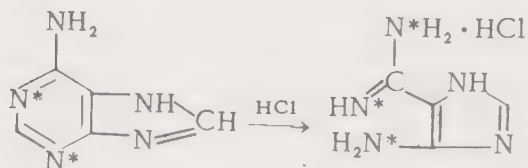
not apparent. In order to check the competing reactions: (1) chloride anion capture by the carbonium ion and (2) carbonium ion rearrangement, the inverse addition of reagents was employed. A methanolic solution of the diazonium salt was added slowly to an excess of ice-cold diazomethane solution. Several crystalline products indicated the reaction of more than 1 mole of diazomethane per mole of diazonium salt. A colorless compound, $C_8H_7N_3O_2$ (m.p. 155°), isolated in 20% yield, was evidently formed from 1 mole of the salt and 2 moles of diazomethane with the elimination of 1 mole of hydrogen chloride and 2 moles of nitrogen. Since the compound had an ultraviolet spectrum similar to *p*-nitroaniline and an infrared spectrum indicating a nitrile group, the probable structure was methyl-4-nitrophenylcyanamide, which is also formed by the reaction of 4-nitrophenylcyanamide with diazomethane. It was considered likely that, in formation of this compound, capture of an anion competes with an intramolecular rearrangement of the active carbonium ion. The mechanism suggested involved a novel sextet inversion which amplifies the known types like the Beckmann, Wolff and Hofmann rearrangements.

2. According to Stollé and Henke-Stark,² treatment of the tetrazole with hot sodium hydroxide solution gives a quantitative yield of 4-nitrophenylcyanamide with loss of nitrogen.

¹R. Huisgen and H. I. Koch, *Naturwissenschaften*, **41**, 16 (1954).

²R. Stollé and F. Henke-Stark, *J. prakt. Chem.*, (2), **124**, 290 (1930).

4(5)-AMINO- N^{15} -5(4)-IMIDAZOLECARBOXAMIDINE- $N^{15}_{1/2}$ HYDROCHLORIDE

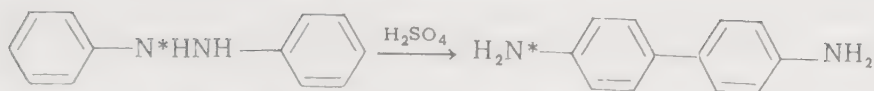


L. F. Cavalieri, J. F. Tinker and G. B. Brown, *J. Am. Chem. Soc.*, **71**, 3976 (1949).

Procedure

Adenine-1,3- N^{15}_2 sulfate, 2.0 g., is heated in a sealed tube with 30 ml. of 6 *N* hydrochloric acid at 150° for 2 hours. The solution is evaporated to dryness, and the residue is extracted with two 10-ml. portions of warm concentrated hydrochloric acid which are combined and filtered. To the filtrate is added 30 ml. of ethanol, and the solution is cooled to induce crystallization. The crystalline product is recrystallized three times by dissolving it in warm concentrated hydrochloric acid (about 2 ml.) and adding alcohol (15 ml.). The yield of pure material is 0.20 g. (10%).

BENZIDINE- N_1^{15}
(4,4'-Biphenyldiamine- N_1^{15})

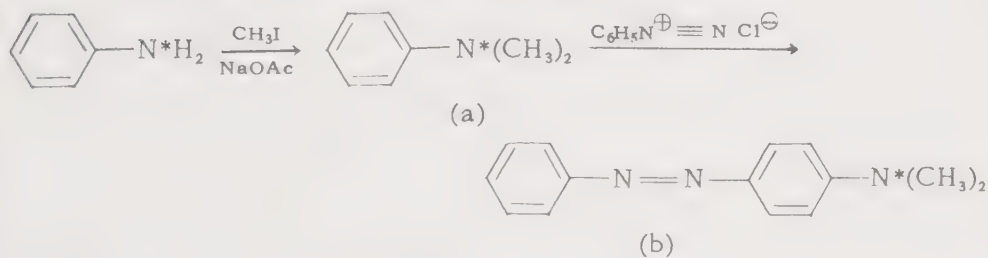


P. F. Holt and B. P. Hughes, J. Chem. Soc., 1955, 98.

Procedure

A solution of 200 mg. of hydrazobenzene- N_1^{15} is poured into 30 ml. of 2 *N* sulfuric acid which contains 30 g. of ice. After the ice melts, the suspension is filtered, and the solid, which is a mixture of benzidine- N_1^{15} and diphenylene- $N_{1/2}^{15}$ (2,4'-biphenyldiamine- $N_{1/2}^{15}$) sulfates, is washed with a little ethanol. The solid is then dissolved in hot, dilute sodium hydroxide solution. When the solution cools, benzidine- N_1^{15} , m.p. 125–126°, separates.

***N,N*-DIMETHYL-4-PHENYLAZOANILINE- N^{15}**



W. S. Fones and J. White, Arch. Biochem., 20, 121 (1949).

A. Procedure

(a) *N,N*-Dimethylaniline- N^{15} . Excess sodium hydroxide solution is added to 11.5 g. of crude aniline- N^{15} hydrochloride, and the free amine is extracted with ether. The solvent is removed, and the residue is heated for 16 hours at 150° in a sealed tube with 40 g. of sodium acetate trihydrate, 32 g. of methyl iodide and 20 ml. of water. The reaction mixture is cooled and made alkaline, and the free amine is removed by steam distillation. The distillate is extracted with solvent, the extract is dried, and the solvent is removed. Distillation of the residue yields 8.5 g. (78.7%) of *N,N*-dimethylaniline- N^{15} , b.p. 190–195°, n_D^{22} 1.5568 (Note 1).

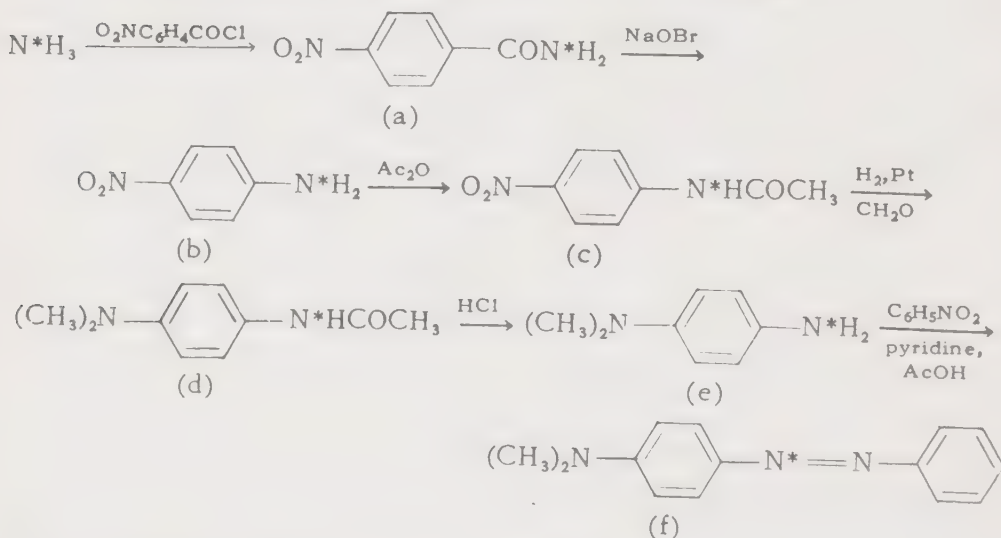
(b) *N,N*-Dimethyl-4-phenylazoaniline- N^{15} . To a stirred solution of 5.7 g. of aniline in 9 ml. of concentrated hydrochloric acid and 50 ml. of water, cooled to 0–5°, there is added dropwise a solution of 4.7 g. of sodium nitrite in 50 ml. of water. After stirring the solution for 0.5 hour, the excess nitrous acid is destroyed by the addition of urea (Note

2). To the cold, stirred solution there is added 7.3 g. of *N,N*-dimethylaniline- N^{15} , and 1 hour later one-half of a solution of 14 g. of sodium acetate trihydrate in 30 ml. of water is added. At the end of the second hour, the rest of this solution is added, and the ice-bath is removed from the reaction vessel. After the reaction mixture is stirred for 4 hours more, it is left overnight. The solid precipitate, 12.4 g., of crude product, m.p. 105–108°, is collected by filtration. Upon recrystallization from 95% ethanol, 11.5 g. (85%) of *N,N*-dimethyl-4-phenylazoaniline- N^{15} is obtained, m.p. 112–114°.

B. Notes

1. Kjeldahl digestion of the residue from the steam distillation gives 8% recovered ammonia- N^{15} .
2. The solution is tested for nitrous acid with starch iodide paper.

N,N-DIMETHYL-4-PHENYLAZO-1- N^{15} -ANILINE



W. S. Fones and J. White, Arch. Biochem., 20, 122 (1949).

A. Procedure

(a) *4-Nitrobenzamide-N-N¹⁵*. Using 19.5 g. of 4-nitrobenzoyl chloride and the ammonia from 11.1 g. of ammonium- N^{15} chloride, 4-nitrobenzamide- $N-N^{15}$ is prepared in a manner analogous to that used for the preparation of benzamide- N^{15} . The crude product is recrystallized from alcohol-water and then water alone to obtain 16.8 g. (99%) of 4-nitrobenzamide- $N-N^{15}$, m.p. 195–198° (Note 1).

(b) *4-Nitroaniline-N-N¹⁵*. To an ice-cold solution of 18 g. of bromine and 12 g. of sodium hydroxide in 100 ml. of water is added 16.8 g. of 4-nitrobenzamide- $N-N^{15}$, and the mixture is shaken thoroughly for about 10

seconds, at 1 minute intervals, during a period of 10 minutes. The resulting slurry is added quickly to a stirred solution of 20 g. of sodium hydroxide in 200 ml. of water, which is heated under reflux. After 30 minutes of heating, the reaction mixture is left overnight. During the reflux period and for 3 hours thereafter, nitrogen gas is bubbled through the reaction mixture and into a sulfuric acid trap (Note 2). The reaction flask is cooled in ice, and the precipitated amine is collected by filtration. The yield of 4-nitroaniline- N - N^{15} is 10.4 g., m.p. $140-147^{\circ}$, which is 75% based on amide used and 89% based on unrecovered ammonia.

(c) 4'-Nitroacetanilide- N - N^{15} . Crude 4-nitroaniline- N - N^{15} , 10.4 g., and 9.0 g. of acetic anhydride in 250 ml. of benzene are heated under reflux until crystallization begins. The reaction mixture is cooled in ice, and the yield of 4'-nitroacetanilide- N - N^{15} , m.p. $212-214^{\circ}$, is 12.3 g. (91%). Concentration of the mother liquor gives 0.7 g. more of crude material, m.p. $200-205^{\circ}$.

(d) 4'-Dimethylaminoacetanilide- N - N^{15} . To 6.5 g. of 4'-nitroacetanilide- N - N^{15} suspended in 190 ml. of 95% ethanol are added 8 ml. of 40% formaldehyde, 3.5 ml. of glacial acetic acid and 0.2 g. of Adams platinum oxide catalyst. During 16 hours of shaking on a Parr apparatus, 5 moles of hydrogen are absorbed. After the catalyst has settled, it is removed by filtration (Note 3).

Removal of the solvent, followed by distillation under reduced pressure, gives 9.8 g. (80.6%) of 4'-dimethylaminoacetanilide- N - N^{15} , b.p. $180-185^{\circ}$ (3 mm.), m.p. $122-126^{\circ}$ (Note 4).

(e) N,N -Dimethyl- p -phenylenediamine- N' - N^{15} , (4-Dimethylaminoaniline- N - N^{15}). 4'-Dimethylaminoacetanilide- N - N^{15} , 9.8 g., is hydrolyzed by heating under reflux for 16 hours in 100 ml. of 3 N hydrochloric acid. The solution is made alkaline, and the amine is extracted with ether. After removal of the solvent, vacuum distillation of the residue yields 6.9 g. (92.3%) of product, b.p. $93-95^{\circ}$ (3 mm.) (Note 5).

(f) N,N -Dimethyl-4-phenylazo-2- N^{15} -aniline. To the solution formed by mixing 5.6 g. of nitrobenzene in 40 ml. of pyridine and 6.3 g. of N,N -dimethyl- p -phenylenediamine- N' - N^{15} in 30 ml. of pyridine is added 5 ml. of glacial acetic acid. After the initial heat of reaction subsides, the solution is heated under reflux for 10 minutes and left at room temperature for 2 hours. The reaction mixture is poured into 500 ml. of ice-water, and the precipitate is collected by filtration. The 9.6 g. of crude product, m.p. $95-105^{\circ}$, is recrystallized from 95% ethanol to obtain 8.3 g. (80%) of material melting at $112-115^{\circ}$.

B. Notes

1. From the mother liquors, 5.5 g. of ammonium- N^{15} chloride is recovered.

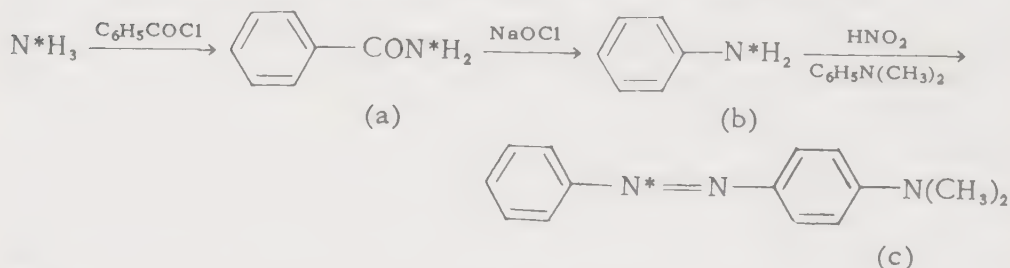
2. In this way, 0.206 g. (14%) of ammonia- N^{15} is recovered.

3. This filtrate was combined with that from a similar experiment in which 5.8 g. of the nitro compound was used.

4. The residue from this distillation is combined with the mother liquors and crude products from the acetylation reaction, and the whole is digested by the Kjeldahl method to recover isotopic nitrogen.

5. Analysis of the aqueous layer, after ether extraction, indicated that 2.8% of the N^{15} was still present.

N,N -DIMETHYL-4-PHENYLAZO-2- N^{15} -ANILINE



W. S. Fones and J. White, Arch. Biochem., 20, 118 (1949).

A. Procedure

(a) *Benzamide- N^{15}* . In a three-necked flask equipped with a stirrer, an inlet tube and an outlet to a boric acid trap (Note 1), is placed 12 g. of benzoyl chloride and 250 ml. of ether. The solution is cooled in a Dry Ice-acetone bath, and the ammonia- N^{15} , generated from 8.21 g. of ammonium- N^{15} chloride, is added during 4 hours. The mixture is left at room temperature overnight. The ether is removed by filtration, and the solid is washed with five 50-ml. portions of absolute ethanol. The combined filtrate and ethanol washings are concentrated to incipient crystallization, and 200 ml. of benzene is added. The solution is heated to boiling and filtered, and the residue is washed with three 100-ml. portions of hot benzene (Note 2). The benzene-alcohol solution is concentrated to 100 ml. and, after cooling, the crystalline product is collected on a filter. Further concentration of the filtrate gives another crop of crystals making the total yield 9.0 g. (97.5%) of benzamide- N^{15} , m.p. 121-123°.

(b) *Aniline- N^{15}* . Into a solution of 30 g. of sodium hydroxide in 100 ml. of water mixed with 150 g. of ice is introduced 8.2 g. of chlorine generated by the action of excess concentrated hydrochloric acid on 7.8 g. of potassium permanganate. To the cold stirred solution is added 12.4 g. of benzamide- N^{15} , and the reaction mixture is heated quickly to boiling. Heating, under reflux, is continued for 1 hour. After the mixture is cooled in an ice-bath, the amine is extracted with three 150-ml. portions

of ether and dried by filtering the extract through anhydrous sodium sulfate, and aniline- N^{15} hydrochloride is formed by the introduction of anhydrous hydrogen chloride. The yield of crude aniline- N^{15} hydrochloride is 12.0 g. (91%).

(c) *N,N*-Dimethyl-4-phenylazo-2- N^{15} -aniline. To a stirred solution of 6.5 g. of the crude aniline- N^{15} hydrochloride in 9 ml. of concentrated hydrochloric acid and 50 ml. of water, cooled to $0-5^{\circ}$, is added dropwise a solution of 3.9 g. of sodium nitrite in 50 ml. of water. After stirring the solution for 0.5 hour, the excess nitrous acid is destroyed by the addition of urea (Note 3). To the cold, stirred solution is added 9 g. of dimethylaniline, and this is followed 1 hour later by the addition of one-half of a solution of 14 g. of sodium acetate trihydrate in 30 ml. of water. At the end of the second hour, the rest of this solution is added, and the ice-bath is removed from around the reaction vessel. After the reaction mixture is stirred for 4 hours more, it is left overnight. The solid precipitate, collected by filtration, weighs 10.7 g. (95.5%), m.p. $113-116^{\circ}$. After recrystallization from ethanol the product melts at $114-117^{\circ}$.

B. Notes

1. For the collection of unreacted ammonia- N^{15} .
2. This residue plus that from the alcohol washings amounts to 3.95 g. (96.5%) of recovered ammonium- N^{15} chloride.
3. The solution is tested for nitrous acid with starch iodide paper.

C. Other Preparations

Benzamide- N^{15} has been prepared from ammonia- N^{15} and benzoyl chloride.^{1,2} Aniline- N^{15} has been prepared by the Hofmann degradation using sodium hypobromite^{1,2} and by thermal decarboxylation of anthranilic- N^{15} acid.³

Benzamide- N^{15} and aniline- N^{15} have been prepared⁴ by a modification of the procedure described; a solution of 0.8 g. of ammonium- N^{15} nitrate was cooled in ice and treated with sodium hydroxide solution, and the resulting solution of ammonia- N^{15} was shaken with a chloroform solution of 1.5 g. of benzoyl chloride. The yield of benzamide- N^{15} was 1.1 g.

Aniline- N^{15} has also been prepared,⁵ in 94.5% yield, by the reduction of nitrobenzene- N^{15} with zinc and hydrochloric acid.

According to the work of Brodskii,² heating of mixtures of primary amine hydrochloride salts results in the formation of secondary amines. At $230-240^{\circ}$, *N*-phenyl-1-naphthylamine- N^{15} was prepared from 1-naphthylamine and aniline- N^{15} ; *N*-phenylbenzamide- N^{15} was obtained from benzamide and aniline- N^{15} ; and, in the formation of diphenylamine- N^{15} from aniline- N^{15} hydrochloride and aniline, the isotopic nitrogen was equally distributed between the reaction products.

¹C. F. H. Allen and C. V. Wilson, *J. Am. Chem. Soc.*, **65**, 612 (1943).

²A. I. Brodskii, B. A. Geller and R. Yu. Sheinfain, *Doklady Akad. Nauk. S.S.S.R.*, **95**, 273 (1954); through *Chem. Abstracts*, **49**, 3862 (1955).

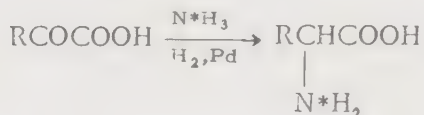
³P. F. Holt and B. I. Bullock, *J. Chem. Soc.*, **1950**, 2310.

⁴G. A. Swan and P. Kelly, *ibid.*, **1954**, 416.

⁵K. Clusius and H. Craubner, *Helv. Chim. Acta*, **38**, 1060 (1955).

2-AMINO-N¹⁵ ACIDS

A GENERAL PROCEDURE



R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, **127**, 301 (1939).

A. Procedure (Note 1)

The reaction vessel used in this general method of preparing 2-amino acids is a 500-ml. 3-necked flask. The delivery tube from an ammonia-N¹⁵ generator is fitted into the flask with a ground-glass joint and extends under the liquid surface. An exit tube is connected with a gas wash bottle containing dilute sulfuric acid for recovery of unabsorbed ammonia-N¹⁵. In the hydrogenation flask is placed a suspension of 3-4 g. of palladium-black (Note 2) in 95% alcohol. The flask is cooled with Dry Ice and, as the ammonia-N¹⁵ (Note 3) is generated, a slow stream of nitrogen is bubbled through the apparatus. When all the ammonia-N¹⁵ is absorbed, the inlet tube is removed and the tip is rinsed with alcohol. The 2-oxo acid dissolved in water (70 ml. per 0.1 mole of acid) is quickly added to the cold alcoholic ammonia-N¹⁵ solution. The ground-glass joint is then fitted with a new inlet tube, which is connected to the source of hydrogen, and the exit tube is still connected to the gas wash bottle.

The hydrogenation apparatus is designed to permit maintenance of sufficient gas pressure, slightly below atmospheric, for rapid hydrogenation without the use of liquid in the hydrogen reservoir. It consists of a 5-liter gas storage flask and a manometer connected to a simple manifold fitted with stopcocks so that the flask and manifold can be evacuated and filled with hydrogen. With this portion of the apparatus filled with hydrogen, the hydrogenation flask, still cooled with Dry Ice, is flushed with hydrogen. The flask is closed with a glass stopper and, after the reaction mixture warms to room temperature, shaking of the flask is begun (Note 4). During the reaction, the hydrogen pressure is increased from time to time, as necessary.

When the hydrogenation is complete, the excess ammonia- N^{15} is recovered by distillation before isolation of the amino acid. The flask is again cooled with Dry Ice and connected through a condenser to a receiver containing dilute acid. An addition tube, which provides for addition of alcohol and the introduction of nitrogen gas, is fitted to the glass joint. The reaction mixture is heated on a steam-bath, and alcoholic ammonia- N^{15} is distilled into the acid trap until the distillate is neutral (Note 5). Then, enough hot water is added to the flask to dissolve the amino acid, and the catalyst is collected on a filter and washed with water. The amino- N^{15} acid is then isolated from the filtrate.

(a) *Alanine- N^{15} , (2-Aminopropionic- N^{15} Acid)*. Freshly distilled pyruvic acid, 2.2 g., is hydrogenated in the presence of 0.05 mole of ammonia- N^{15} . The yield of alanine- N^{15} , obtained by precipitation with alcohol, is 1.49 g. (68%).

(b) *Phenylalanine- N^{15} , (2-Amino-3-phenylpropionic- N^{15} Acid)*. Phenylpyruvic acid, 1.64 g., prepared¹ by the condensation of benzaldehyde and acetylglycine, is hydrogenated in the presence of 0.02 mole of ammonia- N^{15} . The product crystallizes during hydrogenation. After removal of excess ammonia- N^{15} and the catalyst, 1.38 g. (84%) of phenylalanine- N^{15} is obtained.

(c) *Tyrosine- N^{15} , [2-Amino-3-(4-hydroxyphenyl)propionic- N^{15} Acid]*. 4-Hydroxyphenylpyruvic acid, 6.63 g., prepared according to Dakin,² is hydrogenated in the presence of 0.074 mole of ammonia- N^{15} . The tyrosine precipitates during hydrogenation and, after removal of excess ammonia and the catalyst, 5.51 g. (82.8%) of tyrosine- N^{15} is obtained.

(d) *Norleucine- N^{15} (2-Aminohexanoic- N^{15} Acid)*. 2-Oxohexanoic acid, 1.40 g. (Note 6), is hydrogenated in the presence of 0.02 mole of ammonia- N^{15} . The yield of norleucine- N^{15} , which precipitates during the hydrogenation, is 1.02 g. (73%).

(e) *Aspartic- N^{15} Acid, (2-Aminosuccinic- N^{15} Acid)*. Oxalacetic acid, 2 g., prepared by the procedure of Wohl and Claussner³ is hydrogenated in the presence of 0.45 mole of ammonia- N^{15} . The yield is 0.88 g. (44%) of aspartic- N^{15} acid.

(f) *Glutamic- N^{15} Acid, (2-Aminoglutaric- N^{15} Acid)*. 2-Oxoglutaric acid is prepared according to Neuberg and Ringer.⁴ In the presence of 0.15 mole of ammonia- N^{15} , 7.30 g. of the keto acid is hydrogenated. About 150 ml. of alcohol is added, the mixture is cooled to -60° , and the solution is decanted from the catalyst into a Claisen flask connected to two traps containing dilute sulfuric acid. An excess of barium hydroxide is added to the mixture, and the ammonia is distilled into the traps *in vacuo* at $25-30^{\circ}$. The free amino acid is then isolated from the barium salt after removal of barium as the sulfate. The yield of glutamic- N^{15} acid is 6.19 g. (84.2%).

B. Notes

1. The common synthesis of 2-amino acids in which 2-bromo acids are treated with a large excess of ammonia (50 to 70 moles) is not practicable when using isotopic ammonia. Modifications of two known procedures are described by Schoenheimer and Ratner which employ only equivalent amounts of ammonia- N^{15} . They are: 1) the catalytic hydrogenation of 2-oxo acids in the presence of ammonia,⁵ and 2) the coupling of 2-bromo esters¹ with potassium phthalimide (see glycine- N^{15}).

2. To obtain good yields of 2-amino acids it is necessary to use a large quantity of active palladium, e.g., 3 to 4 g. for the reduction of 0.05 mole of keto acid.

3. In the reduction of monocarboxylic keto acids, 2 equivalents of ammonia must be used, for the dicarboxylic acids, 3 equivalents of ammonia are necessary.

4. The hydrogen uptake can be calculated at any time from the change in pressure in the system, the volume of which is known.

5. Several additions of alcohol are necessary. In the preparation of the monoaminodicarboxylic acids, it is necessary to add a fixed base for complete recovery of ammonia. If the amino acid can be heated in the presence of alkali, as is the case with aspartic acid, 2 equivalents of barium hydroxide are added, and the ammonia is distilled as described. When heating must be avoided, as in the case of glutamic acid, the distillation is carried out under reduced pressure, with the precautions described.

6. The preparation of 2-oxohexanoic acid from 2-bromohexanoic acid is given in detail.⁶

C. Other Preparations

Phenylalanine- N^{15} has been prepared,⁷ according to a modification of the method described, in 71-82% yield on a 40-mmole scale.

2-Aminoglutaric- N^{15} acid (glutamic- N^{15} acid) has been prepared⁸ according to the procedure described but, to avoid any possible contamination with barium ion, was isolated by precipitation at its isoelectric point from an alcohol-water mixture, instead of precipitation as the barium salt. L-Glutamic- N^{15} acid was then prepared by the enzymatic method of Fodor⁹ from DL-2-acetamidoglutaric- N^{15} acid (DL-*N*-acetylglutamic- N^{15} acid) prepared by the method of Knoop and Oesterlin.⁵

2-Aminoglutaric-1,2- C_2^{14} - N^{15} acid (glutamic-1,2- C_2^{14} - N^{15} acid) has also been prepared¹⁰ according to the method described, from 2-oxoglutaric-1,2- C_2^{14} acid.

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³A. Wohl and P. Claussner, *Ber.*, **40**, 2308 (1907).

⁴C. Neuberg and M. Ringer, *Biochem. Z.*, **71**, 228 (1915).

⁵F. Knoop and H. Oesterlin, Z. physiol. Chem., 170, 186 (1927).

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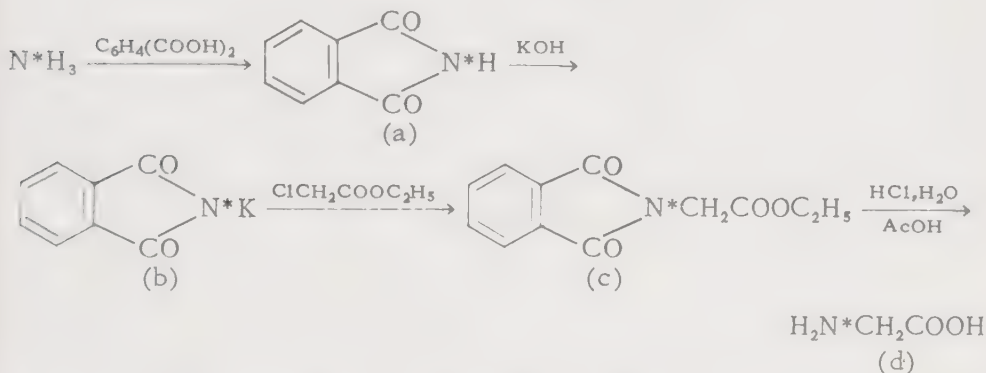
⁷H. D. Baldrige, Jr., W. J. McCarville and J. Sendroy, Jr., Naval Medical Research Institute Report No. 007,099; Nuc. Sci. Abstracts, 7, 3052 (1953).

⁸A. Nisonoff, F. W. Barnes, Jr. and T. Enns, J. Biol. Chem., 204, 957 (1953).

⁹P. J. Fodor, V. E. Price and J. P. Greenstein, J. Biol. Chem., 178, 503 (1949).

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GLYCINE- N^{15}



R. Schoenheimer and S. Ratner, J. Biol. Chem., 127, 301 (1939).

A. Procedure

(a) *Phthalimide- N^{15}* . The ammonia- N^{15} from 0.40 mole of an ammonium- N^{15} salt is distilled with a stream of nitrogen into a suspension of 68 g. of phthalic acid in 200 ml. of water (Note 1). By the time ammonia- N^{15} liberation is complete, the ammonium- N^{15} hydrogen phthalate is all in solution. The solution is transferred to a 1-liter round-bottomed flask which has a neck 75 cm. long and 1.6 cm. wide. At a point 10 cm. from the upper end of the neck, a side arm, 12 mm. i.d., is attached. The side arm is bent in a right angle 25 cm. from the flask and is jacketed with cold water. The lower end of the side arm enters a filtering flask, to the side arm of which is attached a trap containing dilute sulfuric acid. After most of the water has been distilled with a flame, the re-reaction mixture is heated in a metal-bath, and the temperature is slowly raised to 200°, until all the water is removed. The temperature then is raised to 300°. When the vigorous reaction is complete, the flask is cooled, the neck is cut off, and the phthalimide is removed with absolute alcohol. The yield is 56.4 g. (96%).

(b) *Potassium Phthalimide- N^{15}* . The potassium salt of phthalimide- N^{15} is prepared in 92% yield as described by Salzberg and Supniewski.¹ Isotopic nitrogen in by-products is recovered from the mother liquors (Note 2).

(c) *Ethyl 1,3-Dioxo-2-isoindolineacetate-N¹⁵*, (*Ethyl Phthalimidoacetate-N¹⁵*). Potassium phthalimide-N¹⁵, 18.6 g., and 14 g. of ethyl chloroacetate are heated for 2 hours at 150°. The reaction product is extracted with 150 ml. of absolute alcohol and treated with decolorizing carbon. After it is concentrated and cooled, the solution yields 21.2 g. (92%) of ethyl phthalimidoacetate-N¹⁵, m.p. 114° (Note 3).

(d) *Glycine-N¹⁵*. The ester, 15.2 g., is heated under reflux for 2.5 hours with a mixture of 68 ml. each of hydrochloric acid, glacial acetic acid and water. A fraction boiling below 108° is distilled off with the aid of a fractionating column, and heating under reflux is continued for 12 hours. After the solution is cooled, phthalic acid is filtered off, and the filtrate is concentrated to dryness *in vacuo*. The residue is extracted with a small amount of cold water. The solution is diluted, treated with silver carbonate and filtered. The filtrate is treated with hydrogen sulfide, filtered and reduced to a very small volume. The glycine-N¹⁵ is precipitated with alcohol and weighs 5.01 g. (98%).

B. Notes

1. The nitrogen stream is passed from the flask containing the suspension into a gas wash bottle containing sulfuric acid to collect any ammonia not absorbed by the phthalic acid.

2. The alcoholic mother liquors from the preparation of phthalimide and its potassium salt are combined with the contents of the acid traps. An excess of alkali is added, and the ammonia is distilled into sulfuric acid. If the solution is boiled for 4 hours, the recovery of isotopic nitrogen is quantitative.

3. Ammonia-N¹⁵ is recovered from the mother liquor as above.

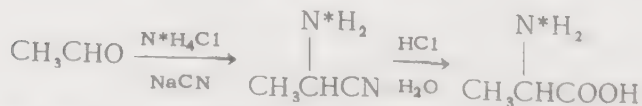
C. Other Preparations

Glycine-2-H₂²-N¹⁵ has been prepared² by exchange with water-H₂². A mixture of 6 g. of glycine-N¹⁵, 200 mg. of platinum oxide catalyst (first reduced with ordinary hydrogen before addition of the glycine-N¹⁵), 10 ml. of water-H₂², and 1 ml. of concentrated hydrochloric acid was shaken in an evacuated, sealed flask at 130° for 21 days.

¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 119.

²D. B. Sprinson and D. Rittenberg, *J. Biol. Chem.*, 184, 405 (1950).

D- AND L-ALANINE-N¹⁵



D. Shemin, *J. Biol. Chem.*, 162, 297 (1946).

A. Procedure (Note 1)

(a) *2-Amino-N¹⁵-propionitrile*. A solution of 1 mole of freshly distilled acetaldehyde (Note 2) dissolved in 100 ml. of ether is placed in a 2-l. bottle and cooled to 5° in an ice-bath. To this solution is added 3.4 moles of ammonium-N¹⁵ chloride, dissolved in 550 ml. of water, followed by an ice-cold solution of 3.1 moles of sodium cyanide in 400 ml. of water. The sodium cyanide solution is added slowly and with frequent cooling to prevent loss of acetaldehyde. After the sodium cyanide solution is added, the bottle is stoppered and shaken for 4 hours at room temperature. The 2-aminopropionitrile is not isolated before hydrolysis.

(b) *Alanine-N¹⁵*. The above mixture is transferred to a 3-l. flask, and 600 ml. of concentrated hydrochloric acid (sp. gr. 1.19) is added (Note 3). The mixture is distilled with a free flame until bumping prevents further heating. It is then evaporated to dryness in an evaporating dish on a steam-bath. The residue remaining in the dish is triturated with 800 ml. of 95% alcohol, which is filtered and distilled on a steam-bath. The last traces of alcohol are removed under vacuum, and the still-warm residue is dissolved in 500 ml. of 95% alcohol containing 2% of hydrochloric acid. The solution is cooled, 200 ml. of ether is added, and the solution is filtered (Note 4). The alcohol and ether are distilled off, and the excess hydrochloric acid is removed under diminished pressure. The residue of alanine-N¹⁵ hydrochloride is dissolved in 1500 ml. of water, 220 g. of yellow lead oxide is added, and the mixture is boiled gently for 1 hour (Note 5). Water is added at intervals to maintain the original volume (Note 6). Upon cooling the mixture lead chloride crystallizes, it is filtered off, and the filtrate is again treated for 1 hour with 100 g. of lead oxide. Then, 20 g. of freshly precipitated lead hydroxide is added slowly, and boiling of the mixture is continued for 20 minutes. The solution is again cooled and filtered (Note 7). The chloride content of the filtrate should now be not more than 0.05–0.075 equivalents (Note 8). The solution is again heated to boiling, and the last of the chloride is removed by addition of the calculated amount of silver oxide. Silver chloride is filtered off, lead is precipitated with hydrogen sulfide, and removal of the lead sulfide leaves a light straw-colored filtrate. This solution is concentrated to about 400 ml., and 600 ml. of 95% alcohol is added. After the solution is thoroughly cooled, the product is collected and washed with 200 ml. of ethanol. The yield of colorless DL-alanine-N¹⁵ is 100–120 g. (Note 9). The total yield is 140–160 g. (52–60%) (Note 10).

Resolution of DL-Alanine-N¹⁵. DL-Alanine-N¹⁵ is resolved into its optical antipodes according to the following procedure of Dunn¹ (Note 11).

(c) *N-Benzoyl-DL-alanine-N¹⁵*, (*2-Benzamidopropionic-N¹⁵ Acid*). To a mixture of 267 g. (3 moles) of DL-alanine, 500 ml. of distilled water

and 175 ml. of saturated sodium hydroxide solution, cooled to 0° , is added rapidly 360 ml. (3.12 moles) of benzoyl chloride, with stirring. Ice and alkali solution (125 ml.) are added as necessary to keep the temperature low and the reaction mixture alkaline to phenolphthalein. Then, 200 ml. of concentrated hydrochloric acid is added, and the mixture is stored overnight in a refrigerator. The suspension of benzoic acid and *N*-benzoyl-DL-alanine is collected and washed with 1.5 l. of cold water and 600 ml. of isopropyl ether. The yield of air-dried product, m.p. $160-161^{\circ}$ (uncor.), is 550 to 557 g. (95 to 97%) (Note 12).

(d) *Strychnine Benzoyl-L(+)-alanine-N¹⁵*. The strychnine benzoyl-L(+)-alanine complex is allowed to crystallize from an aqueous solution containing 2 equivalents of *N*-benzoyl-DL-alanine and 1 equivalent each of potassium hydroxide and strychnine (Note 13). From seven lots (107 to 177 g.) of *N*-benzoyl-DL-alanine (total, 1064 g.) is obtained 1239.7 g. (79.9%) of recrystallized L-strychnine *N*-benzoyl-L(+)-alanine which, according to Pope,⁴ is the dihydrate; $[\alpha]_D^{24} -10.45^{\circ}$ in water.

(e) *Brucine N-Benzoyl-D(-)-alanine-N¹⁵*. The mother liquor from which the strychnine *N*-benzoyl-L(+)-alanine has been removed is evaporated to about one-half its volume. The residual solution is made strongly basic with 3 *N* sodium hydroxide; the mixture is refrigerated overnight, and the suspension of strychnine is collected on a filter and washed with water. The filtrate is acidified to Congo red with concentrated hydrochloric acid, and the precipitate of crude *N*-benzoyl-D(-)-alanine, which forms immediately, is removed by filtration. After two additional crops of crystals are obtained from the filtrate, the yield of crude *N*-benzoyl-D(-)-alanine is 443.2 g. (83%).

To the crude product (2.06 moles, if 90% pure), dissolved in 4.5 l. of boiling water, is added 820 g. (2.08 moles) of anhydrous L-brucine and 12.9 g. (0.23 mole) of potassium hydroxide dissolved in 100 ml. of water. The resulting solution is cooled, seeded and refrigerated for 36 hours. The crystalline salt is collected, washed twice with ice-cold water and dried at 50° . The yield of brucine *N*-benzoyl-D(-)-alanine $\cdot 4.5 \text{ H}_2\text{O}$ (Note 14) is 1059 g. (69.1% based on 443.2 g. of crude *N*-benzoyl-D(-)-alanine and 71% based on 1064 g. of *N*-benzoyl-DL-alanine). After the crude product, m.p. $84-86^{\circ}$ (uncor.), is recrystallized twice from water, the yield of product, m.p. $86-88^{\circ}$ (uncor.), is 890 g. (84% recovery) (Note 15); $[\alpha]_D^{23} -26.53^{\circ}$.

(f) *N-Benzoyl-L(+)-alanine-N¹⁵*. A hot aqueous solution containing 1120.9 g. (1.99 moles) of strychnine benzoyl-L(+)-alanine is made strongly alkaline with 3 *N* sodium hydroxide. The mixture is cooled, and the suspension of strychnine is collected on a filter immediately. The filtrate is acidified with concentrated hydrochloric acid, seeded and refrigerated overnight. The crystalline product is collected on a filter, washed and dried at 50° . The yield of Crude *N*-benzoyl-L(+)-alanine,

m.p. 136–139° (uncor.), is 379.7 g. (99%). The crude product is suspended in 2.5 l. of water at 40°, 121 ml. of concentrated sodium hydroxide solution and 9 g. of activated carbon are added, and the solution is stirred for 10 minutes. After the carbon is filtered off and washed, 215 ml. of concentrated hydrochloric acid is added to the filtrate, which is kept overnight. The crystalline product is collected, washed with water, sucked as dry as possible on the filter and dried at 50°. The yield of recrystallized *N*-benzoyl-L(+)-alanine, m.p. 136–138° (uncor.), from 326.3 g. of crude product is 321.5 g. (98.5%) (Note 16); $[\alpha]_D^{22.5} + 33.4^\circ$ in 1 *N* sodium hydroxide.

(g) *N*-Benzoyl-D(-)-alanine-*N*¹⁵. To a solution of 1337 g. (2.00 moles) of L-brucine *N*-benzoyl-D(-)-alanine·4.5 H₂O, dissolved in 5 l. of water, is added 480 ml. of saturated sodium hydroxide solution. After the mixture is stirred for 10 minutes and cooled to room temperature, the suspension of brucine, which formed rapidly, is collected and washed with 0.5 *N* sodium hydroxide solution. The combined filtrate and washings are acidified to Congo red with concentrated hydrochloric acid (340 ml.) and refrigerated overnight. The crystalline product is collected, washed with cold water and dried at 50° for 2 days. The yield of crude *N*-benzoyl-D(-)-alanine is 364.9 g. (94.5%); $[\alpha]_D^{25} - 32.5^\circ$ in 1.05 *N* sodium hydroxide.

(h) *L*(+)-Alanine-*N*¹⁵. A suspension of 292.5 g. (1.51 moles) of *N*-benzoyl-L(+)-alanine in 1.46 l. of 6*N* hydrochloric acid is heated for 4.5 hours on a boiling water-bath. The mixture is cooled overnight in the refrigerator, 1 l. of distilled water is added, and the solution is filtered (Note 17). The acidic filtrate is concentrated to dryness *in vacuo* and 300 ml. of water is added and evaporated *in vacuo*; this process is repeated twice more. The residual L(+)-alanine hydrochloride is dissolved in a mixture of 400 ml. of distilled water, 89 ml. of concentrated ammonium hydroxide and 293 ml. of 95% ethanol and refrigerated overnight. The crystalline product is collected and washed with 95% ethanol until the washings are free of chloride. The total yield of L(+)-alanine, dried at 50°, is 74.8 g. (64%); $[\alpha]_D^{25} + 13.84^\circ$ in 5.97 *N* hydrochloric acid (first crop of 65.5 g.). The L(+)-alanine, 72 g., is recrystallized by dissolution in 214 ml. of boiling water, followed by the addition of 520 ml. of 95% ethanol. The recovery of dried product is 66.9 g. (93%); $[\alpha]_D^{25} + 13.83^\circ$, in 6.08 *N* hydrochloric acid.

(i) *D*(-)-Alanine-*N*¹⁵. Recrystallized *N*-benzoyl-D(-)-alanine, 230 g. (1.19 moles), is hydrolyzed with concentrated hydrochloric acid. The suspension of benzoic acid is removed by filtration, and D(-)-alanine is isolated from the filtrate essentially by the above method employed in the preparation of L(+)-alanine. The yield of crude D(-)-alanine, collected in three crops, is 101.3 g. (95.8%). From 74.7 g. of the crude product, recrystallized from aqueous ethanol, is obtained 69.6 g. (93.2% recovery); $[\alpha]_D^{25} - 13.57^\circ$, in 6.08 *N* hydrochloric acid.

B. Notes

1. 2-Amino-N¹⁵-propionitrile and DL-alanine-N¹⁵ are prepared from acetaldehyde and isotopic ammonium chloride by a suitable modification of the Strecker² synthesis, as described by Kendall and McKenzie.³

2. Acetaldehyde is conveniently prepared by distillation from paraldehyde in the presence of a trace of sulfuric acid; an efficient fractionating column should be used.

3. Since hydrogen cyanide is evolved, the hydrochloric acid should be added with caution in a hood.

4. This treatment should remove all but the last traces of ammonium chloride and sodium chloride.

5. Use of a metal pail for the lead oxide treatment is recommended.

6. The volume of the solution must be kept large during the treatment with lead oxide, as lead chloride will not crystallize from concentrated alanine solutions.

7. If ammonium salts are still present another treatment with lead oxide is necessary.

8. An aliquot of the solution is titrated with silver nitrate by the Volhard method. The result of the titration is used in calculating the amount of silver oxide which must be added.

9. Additional product may be obtained by concentrating the mother liquor to a volume of about 100 ml., adding 250 ml. of alcohol and cooling the solution to 0°.

10. The product is sufficiently pure for most purposes. It may be recrystallized by dissolution in a minimal amount of hot water and the addition of two volumes of alcohol.

11. The method described by Pope and Gibson⁴ was adopted by Dunn.¹ In this procedure, the strychnine salt of *N*-benzoyl-L(+)-alanine and the brucine salt of *N*-benzoyl-D(-)-alanine are isolated in the order given. According to Dunn,¹ the precipitation of these complexes in the reverse order, according to the original procedure of Fischer,⁵ is not as satisfactory as the procedure of Pope and Gibson,⁴ since the strychnine salt of *N*-benzoyl-L(+)-alanine is much less soluble in water than the brucine salt of *N*-benzoyl-D(-)-alanine. For a bibliography of references regarding the chemical resolution of DL-alanine, see Dunn.¹

12. According to Pope and Gibson⁴ the melting point is 160° (uncor.); Fischer⁵ found 162–163° (uncor.) and 165–166° (cor.).

13. In agreement with the results of Levy and Palmer⁶ it was found that this ratio of reactants is as effective and is more economical of strychnine than the procedure of Fischer⁵ as modified by Pacsu and Mullen.⁷ The latter workers crystallized strychnine *N*-benzoyl-L(+)-alanine from an aqueous solution containing equivalent quantities of *N*-benzoyl-DL-alanine and strychnine.

14. This molecular formula is according to Pope.⁴

15. A product recrystallized four times by Pope and Gibson⁴ melted at 89–91°.

16. The titration equivalent of the product was 189.2 (theory, 193.1), and its moisture content was 1.5%. The pure substance melts at 147–148° (uncor.) or at 150–151° (cor.), according to Fischer.⁵

17. After benzoic acid was extracted from the precipitate on the filter, about 40 g. of unchanged *N*-benzoyl-L-(+)-alanine was recovered.

¹M. S. Dunn, M. P. Stoddard, L. B. Rubin and R. C. Bovie, *J. Biol. Chem.*, **151**, 241 (1943).

²A. Strecker, *Ann.*, **75**, 29 (1850).

³*Organic Syntheses*, Coll. Vol. I, 2nd. ed., Wiley, New York, 1941, p. 21.

⁴W. J. Pope and C. S. Gibson, *J. Chem. Soc.*, **101**, 939 (1912).

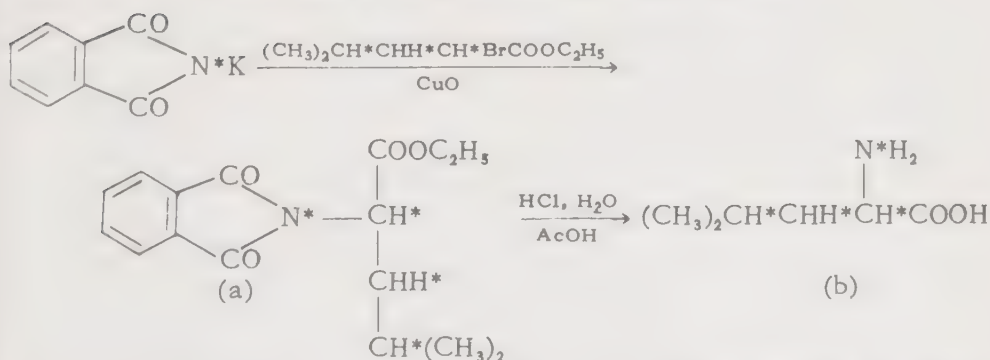
⁵E. Fischer, *Ber.*, **32**, 2451 (1899).

⁶M. Levy and A. H. Palmer, *J. Biol. Chem.*, **146**, 493 (1942).

⁷E. Pacsu and J. W. Mullen, *2nd, ibid.*, **136**, 335 (1940).

LEUCINE-2,3,4- H^2_3 - N^{15}

(2-Amino-4-methylvaleric-2,3,4- H^2_3 - N^{15} Acid)



D. B. Sprinson and D. Rittenberg, *J. Biol. Chem.*, **184**, 405 (1950); R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, **127**, 301 (1939).

A. Procedure

(a) Ethyl α -(2-Methylpropyl-1,2- H^2_2)-1,3-dioxo-2-isoindolylacetate- α - H^2 - N^{15} . Cupric oxide, 8.0 g., and 49.5 g. of potassium phthalimide- N^{15} are ground together in a mortar. This mixture is heated under reflux with 66 g. (0.30 mole) of ethyl 2-bromo-4-methylvalerate-2,3,4- H^2_3 for 3 3/4 hours, using a metal bath at 200°. The resultant mixture is exhaustively extracted with hot ligroin; then the ligroin and unchanged bromo ester are removed by distillation. The residual oil consists chiefly of the desired ester.

(b) DL-Leucine-2,3,4- H^2_3 - N^{15} , (DL-2-Amino-4-methylvaleric-2,3,4- H^2_3 - N^{15}

Acid). The above ester, without further purification, is hydrolyzed by heating under reflux with a mixture of 130 ml. each of acetic acid, concentrated hydrochloric acid and water. The condenser is then replaced by a fractionating column, and the homogenous mixture is slowly distilled until the vapor temperature has risen to 105°. Heating of the residual solution is resumed for 10 hours longer. When the mixture has cooled, phthalic acid is removed by filtration (Note 1), and the filtrate is concentrated, *in vacuo*, to a small volume. A second crop of phthalic acid is removed, and free DL-leucine-2,3,4- $H_3^2-N^{15}$ (Note 2) is isolated from the filtrate after the latter is treated with silver carbonate to remove chloride ions. The yield is 25.4 g. (72%) (Note 3).

(c) *L-Leucine-2,3,4- $H_3^2-N^{15}$* , (*L-2-Amino-4-methylvaleric-2,3,4- $H_3^2-N^{15}$ Acid*). Using an adaptation of the procedure of Schoenheimer,¹ a mixture of 12.7 g. of DL-leucine-2,3,4- $H_3^2-N^{15}$ in 53 ml. of 98% formic acid is heated to 70°, and 18.5 ml. of acetic anhydride is added slowly, with stirring, during 30 minutes (Note 4). Heating is continued for 30 minutes more, 100 ml. of water is added, and the mixture is concentrated to dryness. The residue is dissolved in 80 ml. of ethyl acetate, and the product is precipitated by the addition of petroleum ether (Note 5).

The *N*-formyl-DL-leucine-2,3,4- $H_3^2-N^{15}$ is dissolved in 1 l. of absolute ethanol, 33.4 g. of anhydrous brucine is added, and the solution is heated and then kept at 0° for 24 hours. The crystalline brucine salt of *N*-formyl-D-leucine-2,3,4- $H_3^2-N^{15}$ is collected and washed with 125 ml. of cold alcohol (Note 6). For recovery of free L-leucine-2,3,4- $H_3^2-N^{15}$, the filtrate is concentrated to dryness *in vacuo*. The residue is redissolved in 115 ml. of water; 45.3 ml. of 1 *N* sodium hydroxide is added with cooling, and the precipitate formed is collected and washed with 25 ml. of ice-cold water. The filtrate is rapidly extracted ten times with 25-ml. portions of cold chloroform and once with ether. To the aqueous solution is added sufficient hydrochloric acid to make the solution 1 *N* with respect to hydrochloric acid; it is then heated for 2 hours on a water-bath and evaporated to dryness *in vacuo*. The residue is dissolved in alcohol, filtered to remove sodium chloride and evaporated to dryness. The residual L-leucine-2,3,4- $H_3^2-N^{15}$ hydrochloride is dissolved in a minimal amount of water and adjusted to pH 6 by addition of sodium carbonate. The precipitated product is twice recrystallized from water. The yield of L-leucine-2,3,4- $H_3^2-N^{15}$ is 4.6 g. (72.5%); $[\alpha]_D^{25} +14.2^\circ$ (3.60% in 20% HCl) (Note 7).

(d) *D-Leucine-2,3,4- $H_3^2-N^{15}$* , (*D-2-Amino-4-methylvaleric-2,3,4- $H_3^2-N^{15}$ Acid*). According to Ratner,² the D-isomer is obtained from the alcohol-insoluble brucine salt of *N*-formyl-D-leucine-2,3,4- $H_3^2-N^{15}$ mentioned above. After removal of brucine the D-isomer is recovered in the same manner as the L-isomer, already described.

B. Notes

1. In working up the hydrolysate of the ethyl ester, there is a tendency for leucine to crystallize with phthalic acid after excess hydrochloric acid has been removed *in vacuo*. It is then necessary to extract the phthalic acid residues with dilute hydrochloric acid solution in order to redissolve all of the amino acid.

2. Degradative procedures are described by Sprinson and Rittenberg for determination of the deuterium concentrations at the 2, 3, and 4 positions.

3. The N^{15} from mother liquors of the amino acid preparation is recovered quantitatively as ammonia by subjecting the residues to the Kjeldahl procedure.

4. According to the procedure of Schoenheimer,¹ the resolution of DL-leucine is carried out with the brucine salt of the formyl derivative by a combination of the procedures described by Fischer³ for the resolution of DL-leucine and by du Vigneaud⁴ for the resolution of DL-2-amino-4-phenylbutyric acid.

5. Schoenheimer¹ obtained an 87% yield of N-formyl-DL-leucine, m.p. 115–117°.

6. Schoenheimer¹ reported a 97% yield of the insoluble D-formylleucine brucine salt.

7. The specific rotation for pure L-leucine, under these conditions, is reported, as +14.8°; therefore, the labeled L-isomer was about 98% optically pure. For further purification of the two isomers, Schoenheimer¹ has described a "washing out" process.

¹R. Schoenheimer, S. Ratner and D. Rittenberg, *J. Biol. Chem.*, **130**, 703 (1939).

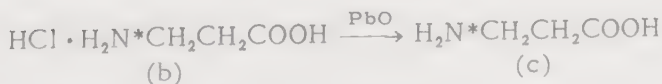
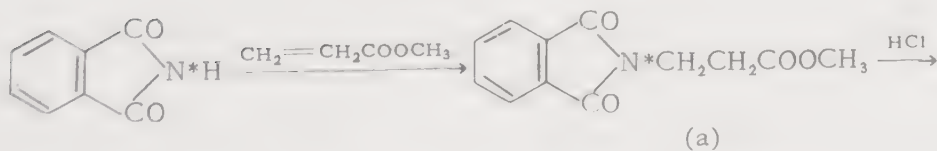
²S. Ratner, R. Schoenheimer and D. Rittenberg, *ibid.*, **134**, 653 (1940).

³E. Fischer and O. Warburg, *Ber.*, **38**, 3997 (1905).

⁴V. du Vigneaud and O. J. Irish, *J. Biol. Chem.*, **122**, 349 (1937–38).

⁵D. W. Thomas and C. Niemann, *ibid.*, **175**, 241 (1948).

β -ALANINE- N^{15}
(3-Aminopropionic- N^{15} Acid)



J. Graff and H. D. Hoberman, *J. Biol. Chem.*, **186**, 369 (1950).

A. Procedure (Note 1)

(a) *Methyl 1,3-Dioxo-2-isoindolinepropionate-N¹⁵*, (*Methyl 3-Phthalimidopropionate-N¹⁵*). β -Alanine-N¹⁵ is synthesized from phthalimide-N¹⁵ and methyl acrylate by a method similar to that of Rodionov and Yartseva.¹

To 14.7 g. of phthalimide, heated to 80°, are added 0.01–0.05 g. of hydroquinone and 5 ml. of a 40% solution of trimethylphenylammonium hydroxide. To the stirred mixture, during 10–15 minutes, are added simultaneously: 50 ml. of methyl acrylate, a few crystals of hydroquinone and 10–15 ml. of the trimethylphenylammonium hydroxide solution. After complete dissolution takes place, the solution is filtered while hot and evaporated at 40 to 60° (Note 2). The residual oil solidifies on standing; the yield of methyl 3-phthalimidopropionate is 91–93%, m.p. 65–77° (Note 3). The pure product, m.p. 73–75°, is obtained by successive recrystallizations from ethanol and benzene.

(b) β -Alanine-N¹⁵ Hydrochloride, (*3-Aminopropionic-N¹⁵ Acid Hydrochloride*). Methyl 3-phthalimidopropionate, 35 g., and 370 ml. of 20% hydrochloric acid are heated under reflux for 9 hours. The solution is cooled and filtered, and the filtrate is evaporated on a steam-bath. The yield of β -alanine hydrochloride, after drying in a desiccator, is 79–83%; m.p. 116–120°, after recrystallization from 96% ethanol.

(c) β -Alanine-N¹⁵, (*3-Aminopropionic-N¹⁵ Acid*). To obtain free β -alanine, 10 g. of the hydrochloride is mixed with 30 g. of lead oxide and 50 ml. of water. The mixture is heated under reflux for 3 minutes and evaporated to dryness on the steam-bath, and the residue is dried at 60–80° for 3 hours. The residue is then extracted with hot water; the extract is treated with hydrogen sulfide, filtered to remove lead sulfide and again evaporated to dryness. The residue is finally dried *in vacuo* over calcium chloride to obtain 5.15 g. (72.3%) of β -alanine; m.p. 173–198°.

B. Notes

1. Experimental details are not given for the isotopic preparation; therefore, the procedures given are from the references cited.

2. The excess methyl acrylate is removed. It would appear desirable to recover this material under reduced pressure, as in the procedure of Galat,² in which acrylonitrile is used.

3. Use of ethyl acrylate gives the corresponding ethyl phthalimidopropionate, (ethyl 1,3-dioxo-2-isoindolinepropionate), m.p. 70–72°.

C. Other Preparations

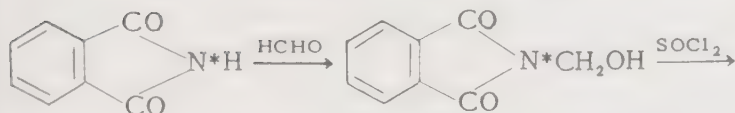
The procedure described is quite similar to that of Galat² who prepared β -alanine in high yield from phthalimide and acrylonitrile. In this

method, the free base is obtained from the hydrochloride by a simple treatment with lithium hydroxide.

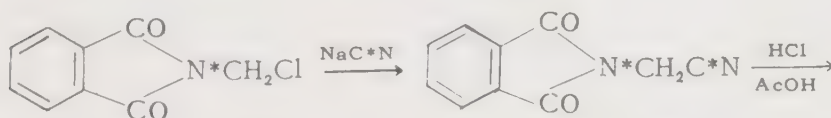
¹Y. M. Rodionov and N. G. Yartseva, Bull. Acad. sci. U.R.S.S., Classe sci. chim. 1948, 251-5; through Chem. Abstracts, 42, 4942 (1948).

²A. Galat, J. Am. Chem. Soc., 67, 1414 (1945).

SERINE-1-C¹³-N¹⁵
(2-Amino-3-hydroxypropionic-1-C¹³-N¹⁵ Acid)

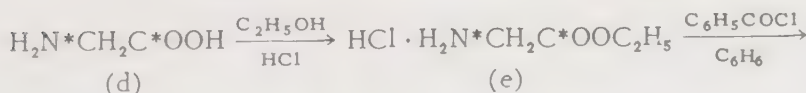


(a)



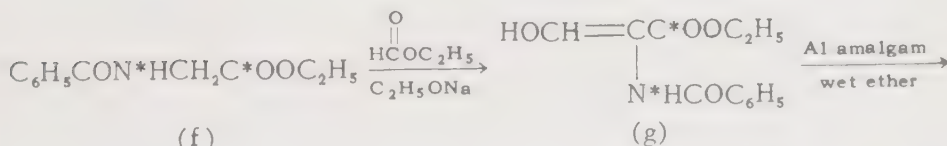
(b)

(c)



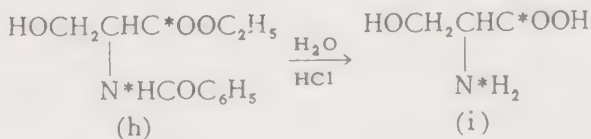
(d)

(e)



(f)

(g)



(h)

(i)

D. Shemin, J. Biol. Chem., 162, 297 (1946).

A. Procedure

(a) *N*-Hydroxymethylphthalimide-N¹⁵, (1,3-Dioxo-2-isoindoline methanol-N¹⁵). Phthalimide-N¹⁵ is condensed with formaldehyde by adaptation of the procedure of Sachs,¹ which is the following. A mixture of 10 g. of phthalimide and 25 ml. of an aqueous 10% formaldehyde solution is heated in a sealed tube at 100° for 1-2 hours. The tube is cooled, opened and heated in a boiling water-bath to remelt the crystalline product, which is poured from the tube. Residual material is washed from the tube with alcohol, and evaporation of the mixture leaves a nearly pure crystalline product, m.p. 139-140°, which is recrystallized from alcohol-toluene mixture.

(b) *N*-Chloromethylphthalimide- N^{15} . *N*-Hydroxymethylphthalimide- N^{15} is converted to *N*-chloromethylphthalimide- N^{15} by treatment with thionyl chloride. When the evolution of sulfur dioxide and hydrogen chloride ceases, excess thionyl chloride is evaporated, and the product is recrystallized from benzene, m.p. 132–133°² (Note 1).

(c) 1,3-Dioxo-2-isoindolineacetonitrile- C^{13} -2- N^{15} , [*N*-(Cyano- C^{13} -methyl)phthalimide- N^{15}]. *N*-chloromethylphthalimide- N^{15} is reacted with sodium cyanide- C^{13} according to the following procedure of Sakami.³

To a solution of sodium cyanide- C^{13} in 25 ml. of acetone-free methanol is added 2.0 g. of *N*-chloromethylphthalimide dissolved in 8 ml. of warm dioxane. After 2 hours the mixture is evaporated to dryness, and the residue is extracted successively with 15, 10 and 5-ml. portions of warm dioxane. The combined extracts are evaporated to dryness, leaving a residue of crude *N*-(cyano- C^{13} -methyl)phthalimide- N^{15} .

(d) Glycine-1- C^{13} - N^{15} . The crude *N*-(cyano- C^{13} -methyl)phthalimide- N^{15} is hydrolyzed by the method of Schoenheimer⁴ according to the procedure of Sakami.³ The material is refluxed with a mixture of 11 ml. of acetic acid, 12 ml. of concentrated hydrochloric acid and 13 ml. of water. After cooling the solution to 0°, phthalic acid is removed by filtration, and the filtrate is evaporated to dryness *in vacuo*. The residue is dissolved in water, chloride is removed with excess silver carbonate, and the precipitate is removed by filtration and washed with water. The combined filtrate and washings are evaporated to 25 ml. and saturated with hydrogen sulfide. After removal of silver sulfide, the filtrate and washings are evaporated to dryness, the residue is taken up in water, and glycine is precipitated by the addition of 95% ethanol and dried; the yield of glycine-1- C^{13} - N^{15} , m.p. 238–239°, is 0.62 g. (81% based on sodium cyanide- C^{13}).

(i) Serine-1- C^{13} - N^{15} . Serine-1- C^{13} - N^{15} is prepared from glycine-1- C^{13} - N^{15} by the same method as that described for the preparation of serine- N^{15} from glycine- N^{15} . The synthesis is *via* the following intermediates:

(e) Glycine-1- C^{13} - N^{15} Ethyl Ester Hydrochloride, (Ethyl Aminoacetate-1- C^{13} - N^{15} Hydrochloride).

(f) Ethyl Hippurate- C^{13} - N^{15} , (Ethyl Benzamidoacetate-1- C^{13} - N^{15}).

(g) Ethyl α -Formylhippurate- C^{13} - N^{15} , (Ethyl 2-Benzamido-2-formylacetate-1- C^{13} - N^{15}).

(h) *N*-Benzoylserine-1- C^{13} - N^{15} Ethyl Ester, (Ethyl 2-Benzamido-3-hydroxypropionate-1- C^{13} - N^{15}).

B. Notes

1. Sakami³ has prepared *N*-chloromethylphthalimide by the reaction of *N*-hydroxymethylphthalimide with excess thionyl chloride for 2 hours at room temperature and 30 minutes on a boiling water-bath. After evapora-

tion of the excess thionyl chloride and recrystallization of the product from toluene, a 77% yield of *N*-chloromethylphthalimide, m.p. 133–134°, was obtained. Recrystallization from dioxane raised the melting point to 134–135°.

¹F. Sachs, Ber., 31, 3230 (1898).

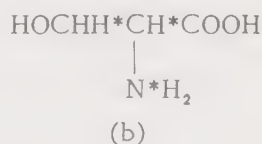
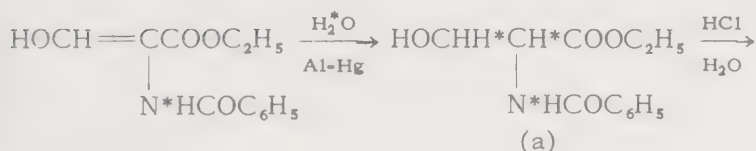
²*Idem*, 31, 1232 (1898).

³W. Sakami, W. F. Evans, and S. Gurin, J. Am. Chem. Soc., 69, 1110 (1947).

⁴R. Schoenheimer and S. Ratner, J. Biol. Chem., 127, 301 (1939).

SERINE-2,3-H₂-N¹⁵

(2-Amino-3-hydroxypropionic-2,3-H₂-N¹⁵ Acid)



D. Elwyn and D. B. Sprinson, J. Biol. Chem., 184, 471 (1950).

A. Procedure

(a) *Ethyl 2-Benzamido-3-hydroxypropionate-2,3-H₂*. According to the procedure of Erlenmeyer,¹ ethyl 2-benzamido-2-formylacetate is dissolved in a 10–15-fold amount of wet ether (Note 1) and treated with aluminum amalgam such that a slight evolution of hydrogen is always evident. From time to time a few drops of water are added to the ether, and the reduction is completed in 4 to 5 days (Note 2). The solution is filtered, dried over sodium sulfate, and distillation of the ether leaves a slightly colored oil which soon solidifies. When the aluminum sludge is extracted with ether, the total yield of the hydroxy ester is 60%. Although the product is easily soluble in ether, alcohol, benzene and ligroin it is best recrystallized from benzene; white needles, m.p. 80°.

(b) *Serine-2,3-H₂-N¹⁵*, (2-Amino-3-hydroxypropionic-2,3-H₂-N¹⁵ Acid). The crude ester is hydrolyzed in 20% hydrochloric acid (Note 3) and heated under reflux for several hours. The benzoic acid formed is extracted from the cooled solution with ether. The aqueous solution of the amino acid is then freed of hydrochloric acid by distillation and finally by exchange on Duolite-A4 resin. The aqueous solution, concentrated to a small volume, is treated with ethanol to effect crystallization of serine-2,3-H₂-N¹⁵ in 28% yield (based on glycine-N¹⁵) (Note 4).

B. Notes

1. In order to introduce deuterium into the compound, this reduction was done with water- H_2^2 by Elwyn and Sprinson.

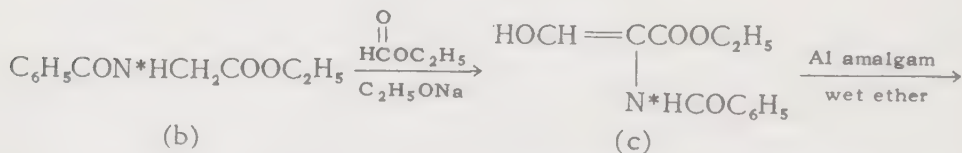
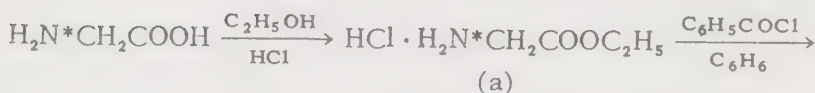
2. The reduction is complete when one drop of the ether solution, diluted with alcohol, does not give color with a drop of ferric chloride solution.

3. Erlenmeyer and Stoop¹ did the hydrolysis in two steps. The ethyl ester linkage was first hydrolyzed at room temperature, during several days, with alcoholic sodium hydroxide. The 2-benzamido-3-hydroxypropionic acid, thus obtained, was then heated under reflux with dilute sulfuric acid to hydrolyze the amide linkage.

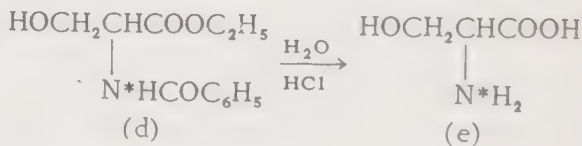
4. DL-Serine-2,3- H_2^2 - N^{15} was resolved by the method of Fischer and Jacobs² to obtain the D- and L- isomers in yields of 43% and 36%, respectively.

¹E. Erlenmeyer and F. Stoop, *Ann.*, 337, 236 (1904).

²E. Fischer and W. A. Jacobs, *Ber.*, 39, 2942 (1906).

SERINE- N^{15} (2-Amino-3-hydroxypropionic- N^{15} Acid)

(b)



D. Stetten, Jr., *J. Biol. Chem.*, 144, 503 (1942).

A. Procedure

(a) *Glycine- N^{15} Ethyl Ester Hydrochloride, (Ethyl Aminoacetate- N^{15} Hydrochloride)*. Dry glycine- N^{15} is esterified with absolute ethanol in the presence of dry hydrogen chloride according to an adaptation of the method of Curtius¹ or Harries,² as follows. Dry hydrogen chloride is passed into a refluxing mixture of 25 g. of glycine in 80 ml. of absolute alcohol until the glycine dissolves completely. The product quickly solidifies upon cooling the syrupy reaction mixture. The crystalline

product is collected, washed with a little alcohol and dried on a water-bath (Notes 1 and 2).

(b) *Ethyl Hippurate-N¹⁵*, (*Ethyl Benzamidoacetate-N¹⁵*). Glycine-N¹⁵ ester hydrochloride is heated under reflux with benzoyl chloride in dry benzene, according to an adaptation of the following procedure.³ A mixture of 20 g. of glycine ester, 22 g. of benzoyl chloride and 100 ml. of benzene is refluxed for 16 hours, or until the evolution of hydrogen chloride ceases. After the solution is filtered and concentrated, the residue is distilled under vacuum; b.p. 206° (26 mm). The distillate is dissolved in ether and crystallized by the addition of petroleum ether. The yield of product, m.p. 60.5°, is 28 g. (95%) (Note 3).

(c) *Ethyl α-Formylhippurate-N¹⁵*, (*Ethyl 2-Benzamido-N¹⁵-2-formylacetate*). Ethyl α-formylhippurate-N¹⁵ is prepared according to an adaptation of the following procedure.⁴ To a solution of 8 g. of sodium in 100–120 ml. of absolute alcohol, in a flask equipped with a reflux condenser and protected from moisture, is added 26 g. of ethyl formate, in small portions with cooling (Note 4). After a few hours, 70 g. of ethyl hippurate is added in solid form with shaking. In a few minutes, the entire mass solidifies to a thick, white paste. After one day, the color of the reaction mixture is yellow and slowly darkens. After 10 days the sodium salt of ethyl formylhippurate is collected and washed thoroughly with absolute ethanol. The salt is then dissolved in water and acidified with hydrochloric acid. The free ester, which appears as an oil, is dissolved in ether and dried. Removal of the ether leaves an 80–90% yield of the oily product, which does not solidify on long standing in vacuum (Note 5).

(d) *N-Benzoylserine-N¹⁵ Ethyl Ester*, (*Ethyl 2-Benzamido-3-hydroxypropionate-N¹⁵*). This compound is prepared according to the following procedure.⁴ The oily ethyl formylhippurate is dissolved in 10–15 times its weight of moist ether. To this solution is added aluminum amalgam, in small portions, at such a rate that hydrogen is steadily evolved. Occasionally, a few drops of water are added also. In this manner, the reduction is completed in 4–5 days (Note 6). The solution is filtered and dried over sodium sulfate, and the ether is distilled. The product remains as a colorless oil which soon crystallizes. More of the product is obtained by extracting the aluminum sludge several times with ether, making the total yield 60% of theoretical. After recrystallization from benzene, the product melts at 80° (Note 7).

(e) *Serine-N¹⁵*, (*2-Amino-3-hydroxypropionic-N¹⁵ Acid*). *N-Benzoylserine-N¹⁵* ethyl ester is refluxed with 7% hydrochloric acid until no more benzoic acid appears upon filtering and cooling a portion of the solution. The solution is cooled, filtered to remove benzoic acid, freed of chloride ions with silver carbonate and again filtered. Serine-N¹⁵ is precipitated

from the aqueous filtrate by the addition of ethanol. The product is collected, washed with ethanol and dried (Note 8). D- and L-Serine-N¹⁵ are prepared⁵ by the resolution of DL-serine-N¹⁵ according to the method of Fischer and Jacobs.⁶ The *p*-nitrobenzoyl derivative of the racemic serine is resolved by forming the quinine salt of the D-component and the brucine salt of the L-component; also see D- and L-alanine-N¹⁵.

B. Notes

1. Concentration of the mother liquor affords more of the product.
2. After recrystallization from absolute ethanol, glycine ethyl ester hydrochloride melts at 142–143°.⁷
3. The ethyl hippurate-N¹⁵, prepared by Stetten, melted at 59–61° after recrystallization from ether-petroleum ether.
4. A white compound separated, and there was a little carbon dioxide evolved.
5. Reduction of the formyl ester to ethyl benzoylserine was best accomplished using the oil. However, it could be further purified by addition of the well-washed salt, in small portions, to a dilute solution of hydrochloric acid. After long contact with the acid, the product slowly solidified to a slightly yellow, hard mass. When the compound was finely ground and freed of a little oil it melted at 50–60°. It was slightly soluble in ether and benzene but easily soluble in alcohol. The alcoholic solution gave an intense red color with ferric chloride solution. The compound did not crystallize from hot benzene, ether or alcohol, but separated as an oil. It was finally crystallized by evaporation of a cold alcoholic solution. After 4 weeks, the colorless needle-like crystals were collected, washed with a little alcohol and dried; m.p. 128°.
6. The end of the reduction is indicated when a drop of the ether solution, diluted with alcohol, does not give a color with ferric chloride solution.
7. The product is soluble in alcohol, benzene, ether and ligroin. Stetten recrystallized the *N*-benzoylserine-N¹⁵ ethyl ester from benzene by the addition of petroleum ether; m.p. 80°.
8. Serine is soluble in water, slightly soluble in alcohol and insoluble in ether, and melts with decomposition at 246°.

C. Other Preparations

Serine-N¹⁵ has been prepared⁴ from glycine-N¹⁵ by a procedure essentially the same as that described, with the exception that the ethyl ester of *N*-benzoylserine-N¹⁵ was hydrolyzed with 20% hydrochloric acid.

Hippuric-N¹⁵ acid, ethyl hippurate-N¹⁵ and ethyl α -formylhippurate-N¹⁵ have been prepared,⁸ in this order, also by procedures like those described.

¹T. Curtis and T. Goebel, J. prakt. Chem., 37, 159 (1888).

²C. Harries and M. Weiss, Ann., 327, 355 (1903).

³H. Franzen, Ber., 42, 2465 (1909).

⁴E. Erlenmeyer, Jr., and F. Stoop, Ann., 337, 236 (1904).

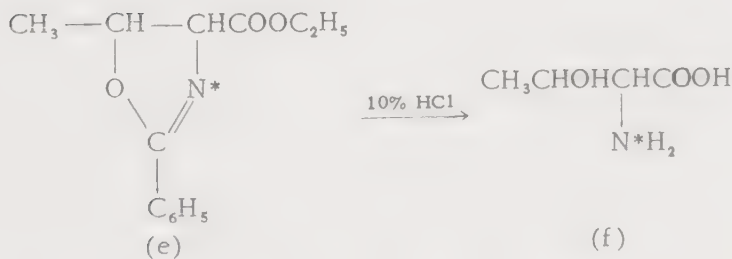
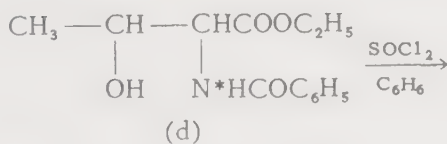
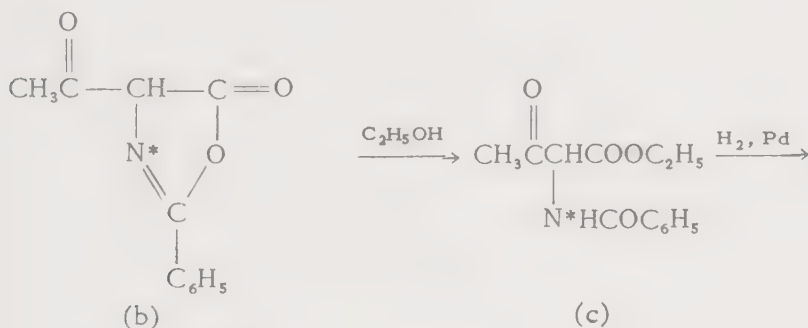
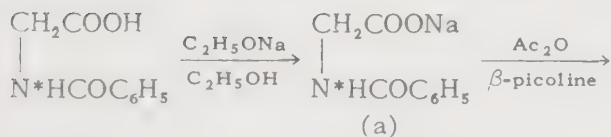
⁵D. Shemin, J. Biol. Chem., 162, 297 (1946).

⁶E. Fischer and W. A. Jacobs, Ber., 39, 2942 (1906).

⁷*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 310.

⁸D. Elwyn and D. B. Sprinson, J. Biol. Chem., 184, 471 (1950).

THREONINE-N¹⁵ (2-Amino-3-hydroxybutyric-N¹⁵ Acid)



H. L. Meltzer and D. B. Sprinson, J. Biol. Chem., 197, 469 (1952).

A. Procedure

The hippuric-N¹⁵ acid, prepared from 50 g. of glycine-N¹⁵, is converted to ethyl 2-benzamidoacetoacetate-N¹⁵ by adaptation of the following procedure of Attenburrow.¹

(a) *Sodium Hippurate-N¹⁵*. To a solution of 447.5 g. of hippuric acid in ethyl alcohol heated under reflux is added dropwise, with stirring, a solution of 57.5 g. of sodium dissolved in 800 ml. of absolute ethanol. The resulting thick paste is collected, and the mother liquor is evaporated to obtain a small amount of product. The combined solids are dried first at 100° and then at 150°. The yield is 488 g. (97.5%) (Note 1).

(b) *4-Acetyl-2-phenyl-2-oxazolin-5-one-N¹⁵*. Sodium hippurate, 878 g., 1330 ml. of β -picoline, and 1310 ml. of acetic anhydride are thoroughly mixed for 2 hours (Note 2) while protected from moisture. After 20 minutes, the mixture attains a temperature of about 40°, then gradually cools. The excess acetic anhydride in the dark green solution is reacted with ethyl alcohol (750 ml.) at 30–35° with stirring and cooling. Distilled water is added (7.5 l.), and the vigorously stirred solution is acidified (Congo red) with dilute hydrochloric acid. The green solid is collected and washed with distilled water (5 l.). The oxazolone is dissolved in 1 *N* sodium hydroxide solution (4.5 l.) at 80° and stirred with 50 g. of charcoal for 15 minutes. The filtrate, cooled at room temperature, is acidified with 6 *N* hydrochloric acid, with vigorous stirring. The slightly impure, yellowish-pink product, dried in a desiccator, weighs 739 g. (83.4%) and melts at 194–195° (dec.).

(c) *Ethyl 2-Benzamidoacetoacetate-N¹⁵*. The oxazolinone, 10 g., is heated under reflux for 2 hours with 100 ml. of absolute ethanol; it slowly dissolves, and the solution is evaporated to dryness under reduced pressure. The quantitative yield of ethyl ester remains as a brown oil. At 150° and 10⁻⁴ mm., the ester distills as a colorless oil with partial reconversion to the oxazolinone (5–10%), which appears in the distillate as a yellow precipitate. The oxazolinone is removed by shaking the ester, dissolved in ether, with sodium bicarbonate solution; the pure ester remains upon evaporation of the ether *in vacuo*.

The ethyl ester, in ethanol solution, may be hydrogenated (Note 3) advantageously without isolation.

(d) *Ethyl 2-Benzamido-3-hydroxybutyric-N¹⁵ Acid*. The ethyl 2-benzamidoacetoacetate in ethyl alcohol is hydrogenated in the presence of 2.0 g. of platinum oxide catalyst at 40 pounds pressure (Note 4). The catalyst is removed by filtration, the solvent is removed *in vacuo*, and the residue, dried by repeated addition and distillation of benzene, is vacuum-distilled.

(e) *Ethyl 5-Methyl-2-phenyl-2-oxazoline-4-carboxylate-N¹⁵ Hydrochloride*. The ethyl 2-benzamido-3-hydroxybutyric-N¹⁵ acid, dissolved in 150 ml. of dry benzene, is treated with 300 ml. of thionyl chloride dropwise during 15 minutes (Note 5). Concentration of the solution and the addition of absolute ether precipitate the crude oxazoline.

(f) *2-Amino-3-hydroxybutyric-N¹⁵ Acid*. The crude oxazoline is heated under reflux with 10% hydrochloric acid² (10 ml. per g.) for 3 hours. The

solution is cooled in an ice-bath, benzoic acid is removed by filtration, and the filtrate is concentrated to dryness with an aspirator. The residue is successively dissolved in water and alcohol and evaporated to dryness (Note 6). The residue is then dissolved in warm anhydrous ethanol, and the solution is made alkaline to litmus with ammonium hydroxide. The yield of crystalline threonine- N^{15} (Note 7) is 28.7 g.

The product is purified² by dissolution in water (4 ml./0.75 g.) and addition of alcohol (9.3 ml.). After 4 hours at 5° the crystalline product is collected, washed with alcohol and ether, and dried. (Note 8).

B. Notes

1. The salt is very hygroscopic.
2. Actually a 3.5-gallon ball mill was used.¹
3. Attenburrow¹ hydrogenated the ester in the presence of Raney nickel at 100° and 75-100 atmospheres. The product, in this case, is the hydroxyester and is hydrolyzed with hydrobromic acid.
4. The hydrogenation is complete when an aliquot of the ethanol solution no longer gives a red color with ferric chloride.
5. Pfister² suggests adding the ester, in portions, to ice-cold thionyl chloride. The resulting solution is kept at room temperature for 2 hours and poured into absolute ether, and the quantitative yield of precipitated gum is crystallized by scratching the flask with a glass rod. The product is purified by dissolution in chloroform and precipitation with absolute ether.
6. Microbiological assay on the aqueous solution at this stage² showed the yield of threonine to be 96.7%.
7. Meltzer and Sprinson indicate the crude product to be a mixture of threonine- N^{15} and allothreonine- N^{15} .
8. Pfister² obtained a 70.1% yield of purified product, m.p. 229-230°.

C. Other Preparations

Shulgin³ prepared threonine- N^{15} from 2-bromo-3-methoxybutyric acid⁴ (the lower melting, 46-52°, isomer) and ammonia- N^{15} using a modification of the procedure of Carter and West.⁴ This procedure, using the lower melting isomer, has the advantage that no allothreonine is formed and the formylation step⁴ is unnecessary. Apparently the 2-amino-3-methoxybutyric- N^{15} acid intermediate was not isolated. The yield of threonine- N^{15} was 0.71 g. (35%) from 3.5 g. of the bromo acid.

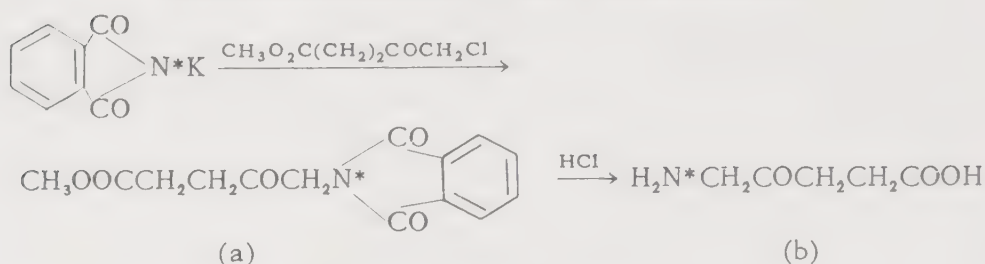
¹J. Attenburrow, D. F. Elliott and G. F. Penny, J. Chem. Soc., 1948, 310.

²K. Pfister, 3rd, C. A. Robinson, A. C. Shabica and M. Tishler, J. Am. Chem. Soc., 71, 1101 (1949).

³A. T. Shulgin, O. G. Lien, Jr., E. M. Gal and D. M. Greenberg, J. Am. Chem. Soc., 74, 2427 (1952).

⁴*Organic Syntheses*, Vol. 20, Wiley, New York, 1940, p. 101.

5-AMINOLEVULINIC-N¹⁵ ACID HYDROCHLORIDE
(5-Amino-4-oxovaleric-N¹⁵ Acid Hydrochloride)



A. Neuberger and J. J. Scott, J. Chem. Soc., 1954, 1820.

A. Procedure

(a) *Methyl 5-Phthalimidolevulinate-N¹⁵*, (*Methyl 4-Oxo-5-phthalimidovalerate-N¹⁵*). To a solution of 10.35 g. (0.0555 mole) of potassium phthalimide-N¹⁵ in 50 ml. of anhydrous dimethylformamide¹ (Note 1) is added 9.137 g. (0.0555 mole) of methyl 5-chloro-4-oxovalerate (methyl 5-chlorolevulinate), with 5 ml. of dimethylformamide for washing. After the mixture is agitated for 0.5 hour, it is warmed at 60° for 1 hour. The reaction mixture is then diluted with water, and the product is extracted with chloroform (Note 2). The product is recrystallized from boiling water (2 l.); the yield is 9.6 g., m.p. 96–97°. An additional 1.2 g. of product, m.p. 96–97°, is obtained by concentrating the mother liquor to 250 ml. and treating it with charcoal; the total yield is 70.5%.

(b) *5-Aminolevulinic-N¹⁵ Acid Hydrochloride*, (*5-Amino-4-oxovaleric N¹⁵ Acid Hydrochloride*). The above imide is refluxed for 8 hours in 7 N hydrochloric acid (10 ml./g. of imide). After the solution is cooled, phthalic acid is removed by filtration, and the mother liquor is concentrated to dryness under vacuum. The product is recrystallized from dry methanol-ethyl acetate; m.p. 145° (dec.) (Note 3).

B. Notes

1. According to Cox and Wame,² traces of water in the dimethylformamide lower the yields from Gabriel condensations in this solvent. The use of dimethylformamide as the solvent in the Gabriel condensation is according to the improved procedure of Sheehan and Bolhofer,³ which is of general application. Potassium phthalimide is appreciably soluble, and the reaction usually goes spontaneously at room temperature. Products of high purity can usually be isolated, and dark-colored reaction tars are completely absent.

2. The dimethylformamide remains in the aqueous phase.³

3. Absorption: λ max. 2665 Å; ϵ max. 23.0, in water; after addition of 2 equivalents of sodium hydroxide and exposure to air, λ max. 2760, ϵ

max. 2000, in accordance with the expected formation at alkaline pH of a 2,5-disubstituted pyrazine.⁴

C. Other Preparations

5-Aminolevulinic- N^{15} acid hydrochloride, m.p. 149–151°, has been prepared:⁵ by the reaction of 3-oxohexanedioic acid^{6–8} with nitrous- N^{15} acid in acetic acid medium followed by reduction of the intermediate nitroso compound with stannous chloride in concentrated hydrochloric acid; from potassium phthalimide- N^{15} and methyl 5-chloro-4-oxovalerate by a procedure similar to that described; and from N^{15} -urocanic acid [4(or 5)-imidazoleacrylic acid] which was hydrogenated with a palladium catalyst to N^{15} -4(or 5)-imidazolepropionic acid, which was converted to its methyl ester and exhaustively benzoylated^{9,10} prior to a two-step hydrolysis with dry methanolic hydrogen chloride and finally 6 *N* hydrochloric acid.

¹F. F. Blicke and Chi-Jung Lu, *J. Am. Chem. Soc.*, **74**, 3933 (1952).

²J. D. Cox and R. J. Warne, *J. Chem. Soc.*, 1951, 1896.

³J. C. Sheehan and W. A. Bolhofer, *J. Am. Chem. Soc.*, **72**, 2786 (1950).

⁴W. N. Hartley and J. J. Dobbie, *J. Chem. Soc.*, 77, 846 (1900).

⁵D. Shemin, C. S. Russell and T. Abramsky, *J. Biol. Chem.*, **215**, 613 (1955).

⁶B. Riegel and W. M. Lilienfeld, *J. Am. Chem. Soc.*, **67**, 1273 (1945).

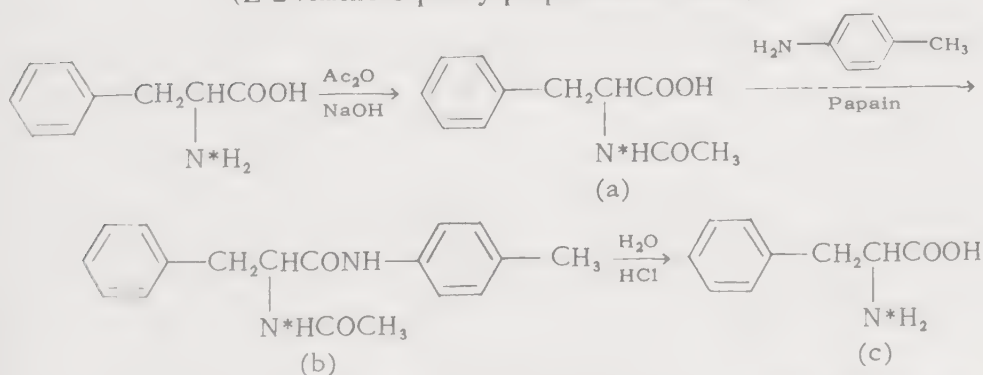
⁷*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 594.

⁸U. Eisner, J. A. Elvidge and R. P. Linstead, *J. Chem. Soc.*, 1950, 2223.

⁹J. N. Ashley and C. R. Harington, *J. Chem. Soc.*, 1930, 2586.

¹⁰C. Tesar and D. Rittenberg, *J. Biol. Chem.*, **170**, 35 (1947).

L-PHENYLALANINE- N^{15} (L-2-Amino-3-phenylpropionic- N^{15} Acid)



H. D. Baldrige, Jr., W. J. McCarville and J. Sendroy, Jr., Naval Medical Research Institute Report No. 007099; *Nuc. Sci. Abstracts*, **7**, 3052 (1953).

A. Procedure

(a) *N*-Acetyl-DL-3-phenylalanine- N^{15} , (DL-2-Acetamido-3-phenylpropionic- N^{15} Acid). A solution of 8.26 g. (50 mmoles) of DL-phenylalanine-

N^{15} in 50 ml. of 2 *N* sodium hydroxide solution (100 mmoles) is cooled to 5°. With rapid stirring, 25 ml. (250 mmoles) of acetic anhydride is added dropwise to the cold solution during about 30 minutes. The temperature of the solution is maintained at 5°, and stirring is continued for 3 hours. The resulting mixture is then concentrated to a thick syrup on a steam-bath under an air stream. The crude product is dissolved in 100 ml. of a 0.5 *M* acetic acid-0.5 *M* sodium acetate buffer solution and kept at room temperature overnight (Note 1).

(b) *N*-Acetyl-*L*-3-phenylalanine- N^{15} *p*-Toluidide, (2-Acetamido- N^{15} -*L*-3-phenyl-*p*-propionotoluidide). To the above solution of *N*-acetyl-DL-3-phenylalanine- N^{15} there is added 0.6 g. (3.8 mmoles) of *L*-cysteine hydrochloride, 5.33 g. (50 mmoles) of *p*-toluidine (Note 2) and an additional 5 ml. of the acetate buffer (Note 3). The mixture is warmed to 50–60° on a steam-bath to effect complete solution and then cooled to 40°. A mixture of 5 g. of papain and 50 ml. of water is stirred for 3 hours and then centrifuged for 15 minutes at 2000 r.p.m. The supernatant solution is added to the acetate-buffered reaction mixture, which is then incubated at 40° for 7 days. Finally the mixture is cooled in a refrigerator for about 2 hours and filtered with vacuum. The crude product is washed with 75–100 ml. of ice-water and air-dried. It is then dissolved, on a steam-bath, in a mixture of 225 ml. of 95% ethanol and 175 ml. of water, filtered and refrigerated overnight. The crystalline product is collected by vacuum filtration and air-dried. The yield of product, m.p. 218° (uncor.) (Note 4), is 5.7–7.0 g. (77–94%).

(c) *L*-Phenylalanine- N^{15} , (*L*-2-Amino-3-phenylpropionic- N^{15} Acid). A solution of 6.98 g. of the above toluidide is refluxed with a mixture of 45 ml. of water for 16 hours. The resulting mixture is concentrated to dryness on a steam-bath under a stream of air, and the residue is dissolved in a solution of 50 ml. of concentrated ammonium hydroxide in 100 ml. of water. The liberated *p*-toluidine is removed by extracting the mixture with four 50-ml. portions of chloroform. The aqueous solution is filtered and again concentrated to dryness on a steam-bath with an air stream. The crude product is recrystallized, first from a mixture of 100 ml. of 95% ethanol and 5 ml. of distilled water, and then from 95% ethanol. The yield of *L*-phenylalanine- N^{15} , $[\alpha]_D^{21}$ –33° (c, 1.5 in water) (Note 5), is 2.10 g. (54%).

B. Notes

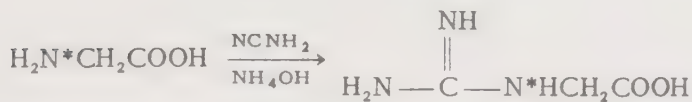
1. This serves to hydrolyze the remaining traces of acetic anhydride.
2. The *p*-toluidine was freshly recrystallized from aqueous ethanol.
3. This is according to the procedure of Huang.¹
4. Huang¹ reported 219°.

5. The value reported in the literature¹ was $[\alpha]_D^{25} -34 \pm 1^\circ$ (c, 2 in water).

¹H. T. Huang and C. Niemann, J. Am. Chem. Soc., 73, 475 (1951).

N-AMIDINOGLYCINE-N¹⁵

(N¹⁵-Glycocyamine)



K. Bloch and R. Schoenheimer, J. Biol. Chem., 138, 182 (1941).

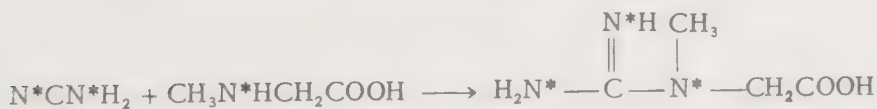
Procedure

N-Amidinoglycine is prepared according to Strecker.¹ To 0.75 g. of glycine-N¹⁵ in 5 ml. of water are added 0.84 g. of cyanamide and 3 drops of concentrated ammonium hydroxide. The mixture is kept at room temperature for 3 days. The crystalline precipitate is collected on a filter and recrystallized from a large volume of water. The yield of N-amidinoglycine-N¹⁵ is 0.75 g.

¹A. Strecker, J. prakt. Chem., 530 (1861).

N¹⁵-CREATINE

[1-Methylguanidino-(1,2)(1,3)-N¹⁵₂-acetic Acid]



K. Bloch, R. Schoenheimer and D. Rittenberg, J. Biol. Chem., 138, 162 (1941).

A. Procedure

Sarcosine-N¹⁵, 0.445 g., is dissolved in 2.0 ml. of water containing 2 drops of concentrated ammonium hydroxide. To this solution, 0.4 g. of cyanamide-N¹⁵ (Note 1), in 3 ml. of water, is added, and the solution is kept at room temperature for 48 hours. The N¹⁵-creatine hydrate is collected on a filter and recrystallized from a small volume of water; yield 0.445 g. (60%).

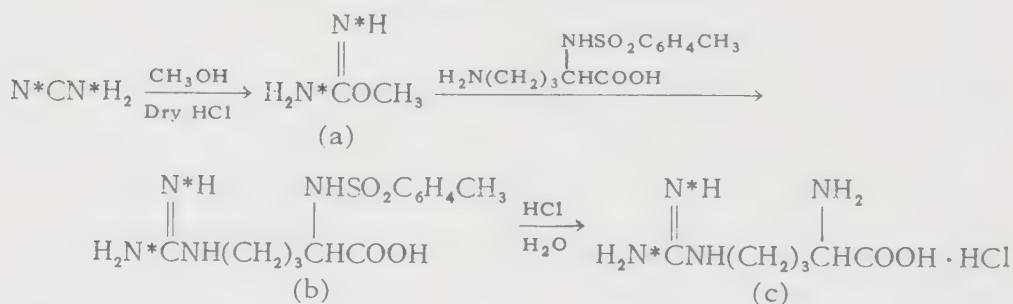
B. Notes

1. According to the mode of preparation, from cyanogen bromide and ammonia-N¹⁵, one of the nitrogen atoms of the cyanamide used should be

isotopic. However, due to tautomerism the two nitrogen atoms in the amidino group of creatine should be functionally equal and indistinguishable with regard to their isotope content.

**L-(+)-2-AMINO-5-GUANIDINO-2,3-N¹⁵_{1/2}-VALERIC
ACID HYDROCHLORIDE**

[N¹⁵_{1/2}-L-(+)-Arginine Hydrochloride]



K. Bloch and R. Schoenheimer, J. Biol. Chem., 138, 167 (1941).

A. Procedure

(a) *2-Methylpseudourea-1,3-N¹⁵_{1/2}*. Cyanamide-N¹⁵_{1/2} is converted into 2-methylpseudourea-1,3-N¹⁵_{1/2} according to an adaptation of the procedure of Stieglitz,¹ which follows. Dry cyanamide, 10.5 g. (0.25 mole), is dissolved in 200 g. of absolute methyl alcohol, and 8.7 g. (0.24 mole) of dry hydrogen chloride is introduced. After two days, no more cyanamide is detectable in the solution. The excess alcohol is distilled off at 40° under reduced pressure, and the residue is then dried in vacuum over sulfuric acid and potassium hydroxide. The yield of 2-methylpseudourea hydrochloride is almost quantitative.

To obtain the free base, 3.2 g. of the hydrochloride, in a small flask, is mixed with 0.5 ml. of water and 20 ml. of alcohol-free ether and cooled to -10°. Into the mixture is introduced powdered potassium hydroxide, in small amounts, until a large excess (20 equivalents) has been added. The ether solution is decanted, and the flask contents are extracted several times with ether. The combined extracts are dried for a short time over anhydrous sodium sulfate, and most of the ether is removed by distillation. The concentrated ether solution is cooled to -15°, and scratching with a glass rod starts crystallization of a nearly quantitative yield of the free 2-methylpseudourea. The free base is collected while cold and is placed on a porous plate over sulfuric acid in a desiccator as quickly as possible. The free base may be further purified (Note 1) by distillation under reduced pressure. At 9 mm. pressure it distills at 82° without decomposition and is collected in an ice-cooled receiver. The

2-methylpseudourea forms a white crystalline mass, m.p. 44–45°, and is a very strong monoacid base which absorbs moisture and carbon dioxide from the air.

(b) 2-(*p*-Toluenesulfonylamido)-5-guanidino-1,3- $N_{1/2}^{15}$ -valeric Acid, [N^2 -(*p*-Toluenesulfonyl)- $N_{1/2}^{15}$ -L(+)-arginine]. A solution of 2.8 g. of 2-methylpseudourea-1,3- $N_{1/2}^{15}$ in 25 ml. of methanol is added to 7.5 g. of N^2 -(*p*-toluenesulfonyl)ornithine,² dissolved in a mixture of 7 ml. of water and 220 ml. of methanol. The mixture is treated with 3 ml. of concentrated ammonium hydroxide and kept in the ice box for 1 week. The crystalline precipitate weighs 4.7 g. (fraction A). On concentration of the filtrate, a second crop, 2.5 g. (fraction B), is obtained. The total yield of crude N^2 -(*p*-toluenesulfonyl)arginine is 7.2 g. (82%).

Fraction A consists mainly of the DL-component (Note 2). Fraction B melts at 256°. After one recrystallization from water, it has a rotation of $[\alpha]_D^{23} -15.1^\circ$ (2% solution in 15% hydrochloric acid) (Note 3). Thus, the more water-soluble reaction product consists of N^2 -(*p*-toluenesulfonyl)-L(+)-arginine; the less soluble fraction is mainly the DL-component.

(c) 2-Amino-5-guanidino-2,3- $N_{1/2}^{15}$ -valeric Acid Hydrochloride, [$N_{1/2}^{15}$ -L(+)-Arginine Monohydrochloride]. For the removal of the *p*-toluenesulfonyl group, the L and DL fractions are hydrolyzed separately by heating with concentrated hydrochloric acid in sealed tubes on a steam-bath for 36 hours. In each instance, the reaction mixture is evaporated to dryness *in vacuo*, the residue is taken up in water, and a slight excess of flavianic acid is added.

From 2.44 g. of N^2 -(*p*-toluenesulfonyl)-L-(+)-arginine, 3.3 g. (90%) of L-(+)-arginine monoflavianate is obtained (Note 4). Flavianic acid is then removed from the L-(+)-arginine salt by suspending the latter in concentrated hydrochloric acid and collecting the free flavianic acid on a filter. The filtrate is diluted with water, treated with carbon and concentrated to dryness *in vacuo*. The residue is taken up in 95% ethanol, and pyridine is added. The yield after recrystallization from water-ethanol is 1.2 g. (77%) of 2-amino-5-guanidino-2,3- $N_{1/2}^{15}$ -valeric acid.

B. Notes

1. The purity of the free base depends upon the purity of the hydrochloride salt obtained.

2. Fraction A displayed a rotation of $[\alpha]_D^{24} -1.8^\circ$ (4% solution in 15% hydrochloric acid). Repeated recrystallizations effected by dissolving the compound in an equivalent amount of hydrochloric acid, followed by neutralization with sodium carbonate, did not accomplish complete removal of the L-isomer. After three crystallizations, the rotation was $[\alpha]_D^{24} -0.7^\circ$. The compound started to decompose slowly at 265°. It is very water-soluble.

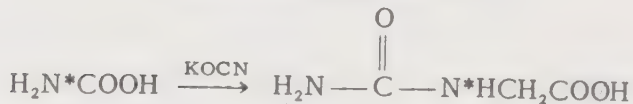
3. This is the rotation displayed by N^2 -(*p*-toluenesulfonyl)-L-(+)-arginine prepared by direct *p*-toluenesulfonation of L-(+)-arginine.

4. From 3.23 g. of N^2 -(*p*-toluenesulfonyl)-DL-arginine there was obtained 4.68 g. (96%) of DL-arginine monoflavianate.

¹J. Stieglitz and R. H. McKee, *Ber.*, 33, 1517 (1900).

²K. Bloch and R. Schoenheimer, *J. Biol. Chem.*, 138, 183 (1941).

HYDANTOIC-3-N¹⁵ ACID
(*N*-Carbamoylglycine-N¹⁵)



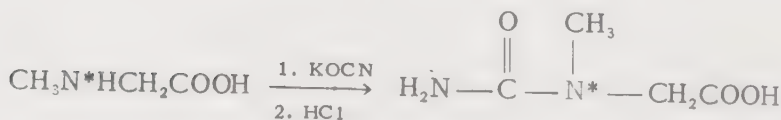
K. Bloch and R. Schoenheimer, *J. Biol. Chem.*, 138, 183 (1941).

Procedure

Hydantoic-3-N¹⁵ acid is prepared according to an adaptation of the procedure of West.¹ Glycine-N¹⁵, 0.525 g., and 0.61 g. of potassium cyanate are dissolved in 20 ml. of water, and the solution is heated on a steam-bath for 1 hour. The solution is filtered, cooled and made acid to Congo red by the addition of concentrated hydrochloric acid. The crystalline precipitate is recrystallized from a small volume of water; the yield is 0.50 g.

¹C. J. West, *J. Biol. Chem.*, 34, 187 (1918).

3-METHYLHYDANTOIC-3-N¹⁵ ACID
(*N*-Carbamoyl-*N*-methylglycine-N¹⁵)

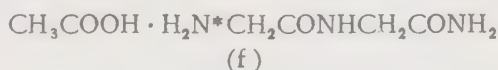
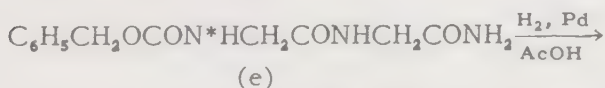
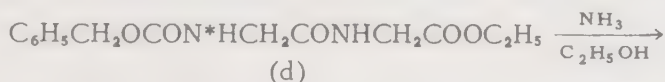
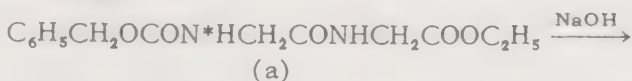


K. Bloch and R. Schoenheimer, *J. Biol. Chem.*, 138, 183 (1941).

Procedure

3-Methylhydantoic-3-N¹⁵ acid is prepared according to the procedure of Salkowski.¹ Sarcosine-N¹⁵, 0.446 g., and 0.41 g. of potassium cyanate in 7 ml. of water are heated on the steam-bath for 5 minutes. The mixture is cooled and acidified with hydrochloric acid. The crystalline precipitate is collected and recrystallized from a small volume of water. The yield of 3-methylhydantoic-3-N¹⁵ acid is 0.33 g.

¹E. Salkowski, *Ber.*, 7, 116 (1874).

N-GLYCYL-N¹⁵-GLYCINE

H. D. Hoberman and D. Stone, J. Biol. Chem., 194, 383 (1952).

A. Procedure

(a) *N*-(*N*-Benzyloxycarbonylglycyl-*N*¹⁵)glycine Ethyl Ester. By a suitable modification of the method of Bergmann,¹⁻⁶ *N*-(*N*-benzyloxycarbonylglycyl-*N*¹⁵)glycine ethyl ester is prepared by condensation of *N*-benzyloxycarbonylglycyl-*N*¹⁵ chloride and glycine ethyl ester⁷ (Note 1).

(b) *N*-(*N*-Benzyloxycarbonylglycyl-*N*¹⁵)glycine. According to a modification of the procedure of Bergmann,³ *N*-(*N*-benzyloxycarbonylglycyl-*N*¹⁵)glycine ethyl ester is shaken for a short time with a 0.1 molar excess of 1 *N* sodium hydroxide in twice its volume of alcohol. A clear solution is obtained; after 45 minutes, the solution is acidified with dilute hydrochloric acid, and the product crystallizes (Note 2).

(c) *N*-Glycyl-*N*¹⁵-glycine. According to the method of Bergmann^{2,6} *N*-(*N*-benzyloxycarbonylglycyl-*N*¹⁵)glycine, in a mixed solvent of aqueous methanol containing one equivalent of acetic acid, is hydrogenated in the presence of palladium-black, in an open vessel. When the evolution of carbon dioxide ceases, the solution is filtered and concentrated to dryness (Note 3).

(d) *N*-Glycylglycine-*N*¹⁵. This compound is prepared from *N*-benzyloxycarbonylglycyl chloride and glycine-*N*¹⁵ ethyl ester according to the above procedures, *via* *N*-(*N*-benzyloxycarbonylglycyl)glycine-*N*¹⁵ ethyl ester and *N*-(*N*-benzyloxycarbonylglycyl)glycine-*N*¹⁵.

(e) *N*-(*N*-Benzyloxycarbonylglycyl-*N*¹⁵)glycinamide. *N*-(*N*-Benzyloxycarbonylglycyl-*N*¹⁵)glycinamide is prepared from *N*-(*N*-benzyloxycarbonylglycyl-*N*¹⁵)glycine ethyl ester according to an adaptation of the procedure of Fruton,⁶ which follows. In 50 ml. of methanol, previously saturated with dry ammonia at 0°, is dissolved 6 g. of *N*-(*N*-benzyloxycarbonyl-

glycyl)glycine ester. After 2 days, the yield of amide which crystallizes is 4.9 g. The product melts at 179-181° after recrystallization from methanol (Note 4).

(f) *N-Glycyl-N¹⁵-glycinamide Acetate*. According to the following procedure of Fruton,⁶ 3 g. of *N*-(*N*-benzyloxycarbonylglycyl)glycinamide is hydrogenated with palladium-black catalyst in methanol containing 0.65 ml. of acetic acid.

The solution is filtered and, upon concentration of the filtrate, 1.9 g. of the amide acetate separates. The product is recrystallized from ethanol-ethyl acetate.

(g) *N*-(*N*-Benzyloxycarbonylglycyl)glycine-N¹⁵ Amide. This compound is prepared from *N*-(*N*-benzyloxycarbonylglycyl)glycine-N¹⁵ ethyl ester as described above for *N*-(*N*-benzyloxycarbonylglycyl-N¹⁵)glycinamide.

(h) *N-Glycylglycine-N¹⁵ Amide Acetate*. *N*-(*N*-Benzyloxycarbonylglycyl)glycine-N¹⁵ amide is treated as in the preparation of *N*-glycyl-N¹⁵-glycinamide acetate.

B. Notes

1. The melting point of *N*-(*N*-benzyloxycarbonylglycyl)glycine ethyl ester is 82-83°.⁶

2. After recrystallization from methanol, *N*-(*N*-benzyloxycarbonylglycyl)glycine melts at 178°.²

3. According to Sheehan,⁸ *N*-glycylglycine decomposes at 215-222° without melting.

4. This compound was also prepared by Hoberman and Stone directly from *N*-benzyloxycarbonylglycyl-N¹⁵ chloride and glycinamide similarly to the preparation of *N*-glycylglycine-N¹⁵.

C. Other Preparations

N-(*N*-Benzyloxycarbonylglycyl)glycine-N¹⁵ and *N*-(*N*-benzyloxycarbonyltryptophyl)glycine-N¹⁵ have been prepared⁹ in yields of about 75% from the reaction of glycine-N¹⁵ with the thiophenol esters of *N*-benzyloxycarbonylglycine and *N*-benzyloxycarbonyltryptophan, respectively.

¹M. Bergmann, *Klin. Wochschr.*, 11, 1569 (1932); *Chem. Abstracts*, 27, 739 (1933).

²M. Bergmann and L. Zervas, *Ber.*, 65B, 1192 (1932).

³M. Bergmann, L. Zervas, J. S. Fruton, F. Schneider and H. Schleich, *J. Biol. Chem.*, 109, 325 (1935).

⁴M. Bergmann, L. Zervas and J. S. Fruton, *ibid.*, 111, 225 (1935); *ibid.*, 115, 593 (1936).

⁵M. Bergmann, L. Zervas and W. F. Ross, *ibid.*, 111, 265 (1935).

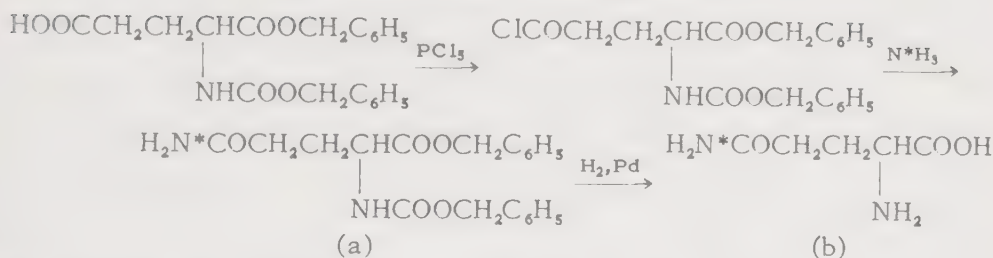
⁶J. S. Fruton and M. Bergmann, *ibid.*, 145, 253 (1942).

⁷*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 310.

⁸J. C. Sheehan and V. S. Frank, *J. Am. Chem. Soc.*, 71, 1856 (1949).

⁹T. Wood and E. R. Roberts, *Biochim. et Biophys. Acta*, 15, 217 (1954).

GLUTAMINE-*N*-N¹⁵
(2-Aminoglutaramic-5-N¹⁵ Acid)



M. Berenbom and J. White, J. Biol. Chem., 182, 5 (1950).

A. Procedure

Glutamine-*N*-N¹⁵, m.p. 186–187° (uncor.), $[\alpha]_D^{23} + 6.2^\circ$ (2% in water), is prepared from glutamic-N¹⁵ acid in 35% yield, based on ammonia-N¹⁵, according to the procedure of Bergmann,¹ which follows (Note 1).

(a) *N*²-Benzyloxycarbonylglutamine-*N*-N¹⁵ Benzyl Ester. A solution of 3.0 g. of 4-benzyloxycarbonyl-4-benzyloxycarbonylaminobutyric acid in 15 ml. of dry ether is cooled in an ice-bath and treated with 1.8 g. of phosphorus pentachloride with agitation (Note 2). The solution is filtered, and the filtrate, protected from moisture, is evaporated to dryness at room temperature and under reduced pressure. The syrupy residue is washed several times with cold, dry petroleum ether. The acid chloride is then dissolved in 25 ml. of dry ether, cooled in an ice-bath and covered over with 50 ml. of an ethereal ammonia solution (Note 3). The resulting needle-like crystals are collected and washed several times with water. The yield of crude product, m.p. 99°, is 2.4 g. After reprecipitation from alcohol with water and recrystallization from a little methanol, the product melts at 123° (cor.).

(b) *Glutamine-N-N¹⁵, (2-Aminoglutaramic-5-N¹⁵ Acid)*. In 15 ml. of pure methyl alcohol is dissolved 1.2 g. of the above benzyl ester. To this solution in an open vessel is added 0.5 ml. of acetic acid and some palladium-black catalyst, and hydrogen is passed through the solution. The hydrogenolysis is complete when carbon dioxide evolution ceases. The solution is filtered, and the filtrate is evaporated to dryness at 25–30° under reduced pressure. The yield of glutamine is 0.4 g. The product is dissolved in water, alcohol is added to turbidity, and the solution is cooled overnight. The yield of purified glutamine, m.p. 184–185°, is 0.26 g.; $[\alpha]_D^{19} + 8.0^\circ$ (in water) (Note 4).

(c) *Ammonium-N¹⁵ Glutamate, (Ammonium-N¹⁵ 2-Aminoglutaramate)*. Ammonia-N¹⁵ is distilled with steam into a suspension of glutamic acid in water. The excess glutamic acid is filtered from the solution of ammonium-N¹⁵ glutamate. The filtrate is then evaporated under reduced pressure (Note 5).

(a) *L*-5-(Ureido-3- N^{15})norvaline Copper Complex, (N_1^{15} -*L*-Citrulline Copper Complex). A solution of 2.5 g. of *L*-ornithine monosulfate in 15 ml. of water is boiled gently for 30 minutes with an excess (1 g.) of cupric

oxide. The deep blue solution, containing the ornithine copper complex, is filtered. To the filtrate is added 3.2 g. of urea, and the solution is concentrated to 10 ml. on a steam-bath (Note 3). The hot concentrated mixture is transferred to a glass tube; the tube is sealed, wrapped in a cloth and heated in a boiling water-bath for 3 hours. The tube, containing a mushy mass of blue citrulline copper complex, is refrigerated overnight and opened. The copper complex is collected, thoroughly washed with water and finally with alcohol. The product, dried in air for 24 hours, weighs 2.03 g. An additional 0.07 g. is obtained by reheating the filtrate for 8 hours, making the total yield 74% (Note 4).

(b) *L*-5-(Ureido-3- N^{15})norvaline, (N_1^{15} -*L*-Citrulline) (Note 5). A suspension of 2.03 g. of citrulline copper complex in 30 ml. of water is treated with hydrogen sulfide. The resultant copper sulfide is very fine but is removed by filtration through a thin, washed layer of infusorial earth (Note 6). The clear solution is concentrated, *in vacuo*, to a small volume, and several volumes of alcohol are added. After the crystallization is well started, a little ether is added, and the solution is cooled overnight. The product is collected, washed with 95% ethanol and dried. The yield of *L*-citrulline, m.p. 218–220° (cor.) with decomposition, is 151 g. (65%, based on ornithine monosulfate). After recrystallization from water by the addition of ethanol, the product melts at 220–221° (cor.) with decomposition (Note 7).

B. Notes

1. The urea- N_1^{15} was prepared from phenyl carbonate and ammonia- N^{15} by the method of Bloch.²

2. The method of Kurtz¹ for synthesizing citrulline from ornithine depends upon the formation of copper complexes by 2- and 3-amino acids and the failure of 4-, 5- and 6-amino acids to give such compounds.³ It was correctly assumed that in the copper complex of ornithine the 2-amino group would be protected, while the 5-amino group would be free to react. The citrulline copper complex, which is of low solubility, precipitates as soon as appreciable amounts are formed. Gornall⁴ used the method to prepare *L*(+)-citrulline from *L*(+)-ornithine hydrochloride.

3. During the concentration, a negligible amount of copper carbonate is precipitated.

4. The purified copper complex of *DL*-citrulline, prepared by Wada,⁵ melted at 257–258°, after three recrystallizations from water.

5. A degradative procedure is described by Hirs and Rittenberg which established that only the carbamoyl group contained excess isotopic nitrogen.

6. When a turbid filtrate is obtained, a drop of dilute hydrochloric acid is added, and the solution is refiltered through a fresh bed of infusorial earth.

7. Gornall⁴ obtained an 80% yield of L-citrulline, m.p. 219.5° after recrystallization, from the copper complex.

C. Other Preparations

C¹⁴-N¹⁵-L-Citrulline, L-[5-(ureido-C¹⁴_{1/1}-3-N¹⁵_{1/1})norvaline], has been prepared⁶ from urea-C¹⁴_{1/1}-N¹⁵_{1/1} and L-ornithine essentially according to the method¹ described.

¹A. C. Kurtz, J. Biol. Chem., 122, 477 (1937-38).

²K. Bloch and R. Schoenheimer, *ibid.*, 138, 167 (1941).

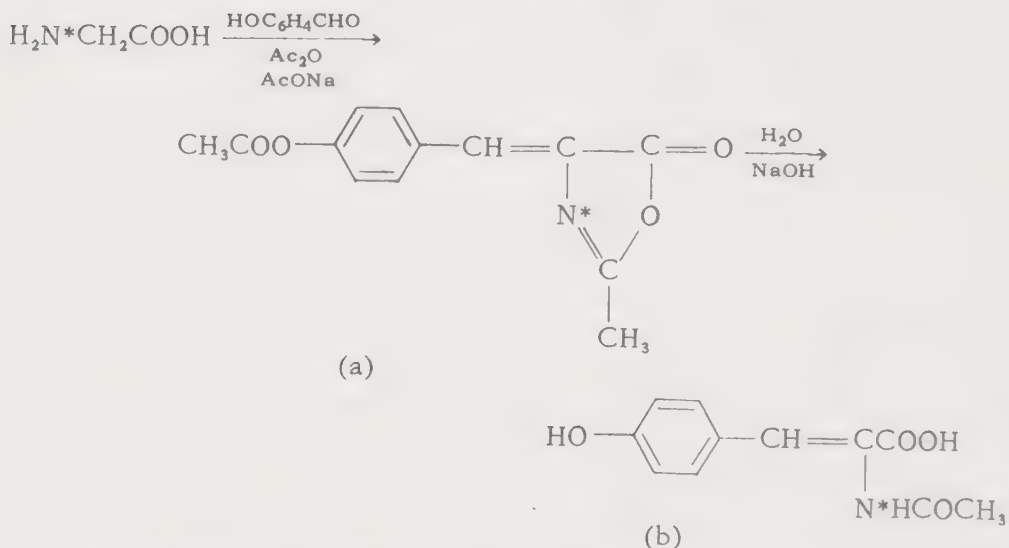
³E. Fischer and G. Zemlén, Ber., 42, 4878 (1909).

⁴A. G. Gornall and A. Hunter, Biochem. J., 33, 170 (1939).

⁵M. Wada, Biochem. Z., 224, 420 (1930).

⁶C. Cooper, R. Wu and D. W. Wilson, J. Biol. Chem., 216, 37 (1955).

α-ACETAMIDO-4-HYDROXYCINNAMIC-N¹⁵ ACID (Acetyldehydrotyrosine-N¹⁵)



H. D. Hoberman and J. S. Fruton, J. Biol. Chem., 182, 127 (1940).

A. Procedure

Acetyldehydrotyrosine-N¹⁵ is prepared by adapting the following procedure of Bergmann.¹

(a) 4-(4-Acetoxybenzylidene)-2-methyl-2-oxazolin-5-one-N¹⁵. Using a slight modification of the procedure of Erlenmeyer,² a mixture of 20 g. of glycine, 34 g. of 4-hydroxybenzaldehyde, 120 ml. of acetic anhydride and 9 g. of fused sodium acetate is heated on a boiling water-bath for 45 minutes. The resulting solution is heated for 30 minutes more in an oil-bath at 115°. After the reaction mixture is cooled to 20° and treated with 135 ml. of water to hydrolyze the excess acetic anhydride, the

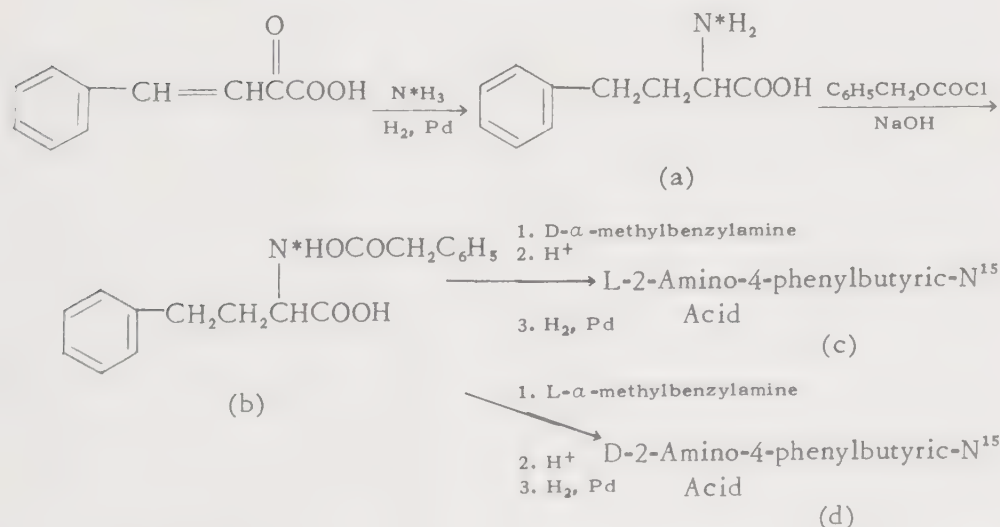
yellow, crystalline oxazalone is collected and washed with water. The yield of crude product is 20-25 g. After recrystallization from ethyl acetate by the addition of petroleum ether, the product melts at 131-132°.

(b) α -Acetamido-4-hydroxycinnamic- N^{15} Acid, (Acetyldehydrotyrosine- N^{15}). A mixture of 0.5 g. of the oxazalone and 7.5 ml. of 1 *N* sodium hydroxide solution is warmed at 60° until a clear solution is obtained. The solution is cooled and neutralized with an equivalent amount of 5 *N* hydrochloric acid. The yellow crystals, which soon appear, are collected and recrystallized from 7 ml. of water. The microscopic prisms of product melt at 150° with bubbling, resolidify and again melt at 202° (uncor.). The product from water contains 1 mole of water of crystallization; it is very soluble in warm alcohol, soluble in warm acetone and slightly soluble in ethyl acetate, benzene and chloroform.

¹M. Bergmann and F. Stern, Ann. 448, 20 (1926).

²E. Erlenmeyer, Jr., and J. T. Halsey, Ann., 307, 139 (1899).

D- and L-2-AMINO-4-PHENYLBUTYRIC- N^{15} ACID



V. du Vigneaud, M. Cohn, G. B. Brown, O. J. Irish, R. Schoenheimer and D. Rittenberg, J. Biol. Chem., 131, 278 (1939).

A. Procedure

(a) *DL*-2-Amino-4-phenylbutyric- N^{15} Acid. Benzylidenepyruvic acid,¹ 15 g., in 200 ml. of 50% alcohol is hydrogenated² in the presence of 2.42 g. of ammonia- N^{15} and 5 g. of palladium catalyst (Note 1). During the hydrogenation, *DL*-2-amino-4-phenylbutyric- N^{15} acid crystallizes. The reaction is stopped when 1.8 moles of hydrogen are absorbed (Note 2). The organic material is extracted from the catalyst with dilute sodium hydroxide solution, which is filtered and acidified with acetic acid. The

precipitate of amino acid is washed with cold water and alcohol (Note 2). The yield of amino acid, recrystallized by extraction with hot water from a thimble, is 10.5 g. (70%) (Note 4).

(b) *DL-2-Benzylloxycarbonylamino-4-phenylbutyric-N¹⁵ Acid*. A solution of 3.7 g. of DL-2-amino-4-phenylbutyric-N¹⁵ acid in 10.3 ml. of 2 *N* sodium hydroxide is cooled in an ice-bath, and 20 ml. of ether is added to the solution. While the mixture is stirred, 4.0 g. of benzyloxycarbonyl chloride³ is added in four portions at 10-minute intervals; the solution is maintained alkaline by the addition of 4 *N* sodium hydroxide solution.

After the solution is stirred continuously for 1 hour, the major portion of the sodium salt of DL-2-benzyloxycarbonylamino-4-phenylbutyric-N¹⁵ acid separates as a third layer. This layer is separated and dissolved in 400 ml. of water. The resulting solution is acidified with hydrochloric acid, and the benzyloxycarbonyl derivative crystallizes as the solution is cooled. An additional quantity of product is obtained by dilution and acidification of the original sodium hydroxide layer. The entire product is recrystallized by dissolution in ammonium hydroxide and reprecipitation by acidification of the solution with hydrochloric acid. The yield is 6.4 g. (98%); m.p. 112°.

Resolution of DL-2-Amino-4-phenylbutyric-N¹⁵ Acid. To 6.4 g. of the above acid, dissolved in 25 ml. of ethyl acetate, is added 2.66 ml. of D- α -methylbenzylamine. The solution is cooled overnight, and 2.14 g. of crude D- α -methylbenzylamine salt of L-2-benzyloxycarbonylamino-4-phenylbutyric-N¹⁵ acid is collected.

After addition of 200 ml. of water to the filtrate, with thorough mixing the solution is made alkaline to phenolphthalein with ammonium hydroxide (Note 5). The ethyl acetate layer, containing the excess D- α -methylbenzylamine, is separated, and the aqueous solution is again extracted with a 10-ml. portion of ethyl acetate. The aqueous solution is then slowly acidified, and the D-2-benzyloxycarbonylamino-4-phenylbutyric-N¹⁵ acid crystallizes when the solution is cooled overnight. Without further recrystallization, the impure (Note 6) acid is dissolved in 20 ml. of ethyl acetate, and 2.0 ml. of L- α -methylbenzylamine is added. The solution is cooled overnight, and 2.4 g. of crude L- α -methylbenzylamine salt of D-2-benzyloxycarbonylamino-4-phenylbutyric-N¹⁵ acid is obtained.

The mother liquor from this fraction is then extracted with dilute ammonium hydroxide, and the mixture of benzyloxycarbonyl derivatives of D- and L-2-amino-4-phenylbutyric-N¹⁵ acids is carried through successive treatments with L- and D- α -methylbenzylamine respectively. This entire process is carried through four times, in all, and a fairly complete separation of the isomers is effected. A total yield of 3.3 g. of the D- α -methylbenzylamine salt of the L-isomer is obtained, and the final yield of L- α -methylbenzylamine salt of the D-isomer is 3.0 g.

After four recrystallizations of the D- α -methylbenzylamine salt of the L-isomer from minimum volumes of hot ethyl acetate, 2.3 g. is obtained

(Note 7). Repeated fractionation of the mother liquors yields an additional 0.35 g. of equal purity. To remove traces of N^{15} -D-isomer which may be present in this salt of the L-isomer, it is "washed out" (Note 8) with some N^{14} -D-isomer. The D- α -methylbenzylamine salt of L-2-benzyloxycarbonylamino-4-phenylbutyric- N^{15} acid, 2.65 g., and 0.10 g. of D-2-benzyloxycarbonylamino-4-phenylbutyric acid are dissolved in 25 ml. of ethyl acetate, and 0.04 ml. of D- α -methylbenzylamine is added. The salt of L-isomer obtained is recrystallized three times from ethyl acetate. The yield of final product, with a rotation of $[\alpha]_D^{20} +19.4^\circ$, is 2.33 g.

(c) *L-2-Amino-4-phenylbutyric- N^{15} Acid*. The 2.33 g. of the D- α -methylbenzylamine salt of L-2-benzyloxycarbonylamino-4-phenylbutyric- N^{15} acid is dissolved in 100 ml. of dilute ammonium hydroxide. The solution is extracted with two portions of ethyl acetate (Note 8) and is then acidified to recover the L-2-benzyloxycarbonylamino-4-phenylbutyric- N^{15} acid. This material is dissolved in 30 ml. of 70% ethanol, and 5.4 ml. of sulfuric acid is added. The benzyloxycarbonyl group is removed by hydrogenolysis with a palladium-black catalyst.³ After the hydrogenation, the solution is neutralized with dilute sodium hydroxide, and the L-2-amino-4-phenylbutyric- N^{15} acid is collected and recrystallized from a minimum of hot water containing a trace of hydrochloric acid; yield, 0.835 g. (45.5%) (Note 9).

(d) *D-2-Amino-4-phenylbutyric- N^{15} Acid*. The 3.0 g. of L- α -methylbenzylamine salt of D-2-benzyloxycarbonylamino-4-phenylbutyric- N^{15} acid is combined with 3.15 g. from a duplicate experiment and recrystallized to a constant rotation of $[\alpha]_D^{20} -19.1^\circ$. The 5.6 g., thus obtained, is mixed with 0.2 g. of L-2-benzyloxycarbonylamino-4-phenylbutyric acid, and after addition of the required amount of L- α -methylbenzylamine the mixture is fractionated. After five recrystallizations and repeated fractionation of the mother liquors, 4.35 g. of product, with a rotation of $[\alpha]_D^{21} -19.3^\circ$, is obtained. The D-2-benzyloxycarbonylamino-4-phenylbutyric- N^{15} acid is recovered from the salt, as previously described, and hydrogenation yields 1.6 g. (44%) of D-2-amino-4-phenylbutyric- N^{15} acid.

B. Notes

1. A simple but effective apparatus and the general method of preparing 2-amino acids from 2-keto acids by hydrogenation in the presence of ammonia are described. Several amino- N^{15} acids are prepared by this method; see alanine- N^{15} .

2. Excess isotopic ammonia is recovered by distilling most of the solvent, with a stream of nitrogen, into dilute sulfuric acid.

3. The alcohol removed a small amount of unchanged benzylidenepyruvic acid.

4. The total recovery of isotopic nitrogen is 98.8%.

5. The D-2-benzyloxycarbonylamino-4-phenylbutyric- N^{15} acid (with some L-isomer) is extracted into the aqueous phase as ammonium salt.

6. Some of the L-isomer is still present.

7. The rotation, $[\alpha]_D^{20} +19.2^\circ$ for a 3% solution in ethyl alcohol, remained constant after the second recrystallization.

8. The principle of the washing-out process is described in detail in the resolution of leucine- N^{15} .⁴

9. Based on the initial sample of the amino acid.

¹E. Friedmann, *Helv. Chim. Acta*, **14**, 783 (1931).

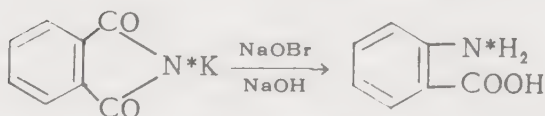
²R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, **127**, 301 (1939).

³M. Bergmann and L. Zervas, *Ber.*, **65B**, 1192 (1932).

⁴R. Schoenheimer, S. Ratner and D. Rittenberg, *J. Biol. Chem.*, **130**, 703 (1939).

ANTHRANILIC- N^{15} ACID

(2-Aminobenzoic- N^{15} Acid)



P. F. Holt and B. I. Bulloch, *J. Chem. Soc.*, 1950, 2310.

A. Procedure (Note 1)

A cold hypobromite solution is prepared by the addition of 0.4 ml. of bromine to 1.2 g. of sodium hydroxide in 5 ml. of water at 0° . To this solution is added 1.0 g. of potassium phthalimide- N^{15} , followed immediately by 1.07 g. of sodium hydroxide in 3.3 ml. of water. The solution is heated for 2 minutes at 80° , then cooled in ice and neutralized by the dropwise addition of hydrochloric acid. The anthranilic- N^{15} acid, precipitated by the addition of 1 ml. of acetic acid, is collected, washed with water and dried *in vacuo* (Note 2).

B. Notes

1. Partridge¹ and Schayer² have also prepared anthranilic- N^{15} acid by the Hofmann degradation of phthalimide- N^{15} using an adaptation of the procedure of Hoogewerff,³ which is described by Weygand.⁴ According to the latter reference, the yield of anthranilic acid, m.p. $144-145^\circ$, was 87% on a 0.1-mole scale.

2. The crude product can be purified⁴ by recrystallization from water or from ligroin. The anthranilic acid retained in the aqueous mother liquor can be precipitated with cupric acetate solution as copper anthranilate.

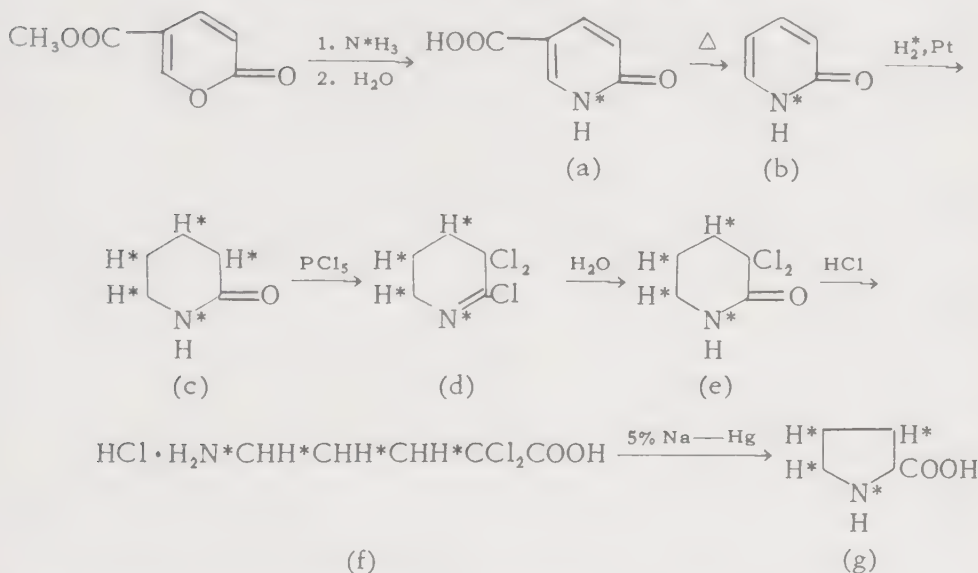
¹C. W. H. Partridge, D. M. Bonner and C. Yanofsky, *J. Biol. Chem.*, **194**, 269 (1952).

²R. W. Schayer, *ibid.*, **187**, 777 (1950).

³S. Hoogewerff and W. A. van Dorp, *Rec. trav. chim.*, 10, 6 (1891).

⁴C. Weygand, *Organic Preparations*, Interscience, New York, 1945, p. 465.

PROLINE-3,4,5-H³-N¹⁵
(2-Pyrrolidinecarboxylic-3,4,5-H³-N¹⁵ Acid)



M. R. Stetten and R. Schoenheimer, *J. Biol. Chem.*, 153, 115 (1944).

A. Procedure

(a) *6-Hydroxynicotinic-N¹⁵ Acid*, (*6-Hydroxy-3-pyridinecarboxylic-N¹⁵ Acid*). Coumalic acid, prepared from malic acid according to von Pechmann,¹ is esterified with methanol, and the ester² is purified by vacuum distillation followed by recrystallization from ligroin.

The ammonia from 17.0 g. of ammonium-N¹⁵ chloride is carried by a stream of nitrogen into a suspension of 22 g. (0.143 mole) of coumalic ester² in 70 ml. of water cooled with ice (Note 1). The ester gradually dissolves and the solution turns yellow as 6-hydroxynicotinic-N¹⁵ acid is formed. The solution of ammonium 6-hydroxynicotinate-N¹⁵ is let warm to room temperature and after about 1 hour is cooled with ice and made alkaline with 70 ml. of 40% sodium hydroxide solution (Note 2). The alkaline solution is boiled for 5 minutes, and nitrogen is passed through for 30 minutes longer in order to collect all the liberated ammonia-N¹⁵ in the acid trap. The solution is cooled and acidified to Congo red indicator with concentrated hydrochloric acid; the precipitate is filtered and washed with cold water. The tan powder so obtained is purified by dissolution in dilute sodium bicarbonate and precipitation with hydrochloric acid to yield 17.7 g. (89%) of 6-hydroxynicotinic-N¹⁵ acid (Note 3).

(b) 2(1H)-Pyridone- N^{15} , (2-Pyridinol- N^{15}). Dry, powdered 6-hydroxynicotinic- N^{15} acid is decarboxylated as described by von Pechmann and Baltzer.³ The dry acid in a large distilling flask is heated a little above its melting point with a metal-bath until a lively evolution of carbon dioxide begins. When the melt is quiescent, it is heated more strongly, and the 2(1H)-pyridone- N^{15} distills as a colorless oil.

From 141.8 g. of 6-hydroxynicotinic- N^{15} acid, treated 20 g. at a time, 89.3 g. (93%) of 2(1H)-pyridone- N^{15} is obtained which is purified by recrystallization from ethyl acetate. The yield of purified product is 77.3 g. (80%), m.p. 106-107°.

(c) 2-Piperidone-3,4,5,6- $H_4^2-N^{15}$. To introduce the deuterium, the 2(1H)-pyridone- N^{15} in glacial acetic acid is shaken at 100° with deuterium gas (99.6 atom per cent H^2) at slightly less than 1 atmosphere of pressure. A platinum catalyst is used in an apparatus described by Rittenberg and Schoenheimer.⁴

In a typical experiment, with 26.0 g. of 2(1H)-pyridone- N^{15} and 2.5 g. of platinum catalyst in 25 ml. of glacial acetic acid, deuterium is consumed as fast as it can be generated (Note 4). Theoretical uptake of deuterium is obtained in about 7 hours. When the hydrogenation is complete, the catalyst is collected on a filter, the acetic acid is evaporated, and the 2-piperidone-3,4,5,6- $H_4^2-N^{15}$ is purified by distillation. The yield of material boiling at 134° (14 mm.) is 22.4 g. (83%).

(d) 5,5,6-Trichloro-2,3,4,5-tetrahydropyridine-2,3,4- $H_3^2-N^{15}$ (Note 5). The trichloro compound is formed by heating freshly distilled 2-piperidone-3,4,5,6- $H_4^2-N^{15}$ with 3 equivalents of phosphorus pentachloride in dry xylene. The reaction mixture is fractionally distilled, and the portion boiling between 55° and 110° at 4-5 mm. is collected (Note 6).

(e) 3,3-Dichloro-2-piperidone-4,5,6- $H_3^2-N^{15}$. Upon addition of water to the trichloropiperidone-4,5,6- $H_3^2-N^{15}$, 3,3-dichloropiperidone-4,5,6- $H_3^2-N^{15}$ is formed immediately.

(f) 5-Amino-2,2-dichloro-3,4,5- $H_3^2-N^{15}$ Acid. The dichloropiperidone is further hydrolyzed by heating under reflux with hydrochloric acid to obtain the hydrochloride of 5-amino-2,2-dichloro-3,4,5- $H_3^2-N^{15}$ acid. The solution is concentrated and made just alkaline to litmus with sodium carbonate. The isotopic aminodichloro-3,4,5- $H_3^2-N^{15}$ acid precipitates in colorless crystals.

(g) Proline-3,4,5- $H_3^2-N^{15}$, (2-Pyrrolidinecarboxylic-3,4,5- $H_3^2-N^{15}$ Acid). The isotopic dichloroamino-3,4,5- $H_3^2-N^{15}$ acid is not isolated but is reduced directly to proline-3,4,5- $H_3^2-N^{15}$. To the ice-cold, stirred suspension of the acid, 5% sodium amalgam is added gradually over a period of several hours. The mixture is evaporated to dryness, and the proline, isolated by extraction with alcohol, is converted to the copper salt (Note 7). The copper salt is decomposed with hydrogen sulfide, and the proline-3,4,5- $H_3^2-N^{15}$ is recrystallized from absolute ethanol.

B. Notes

1. Any ammonia not absorbed by the reaction mixture is caught in a trap containing hydrochloric acid.
2. The yield is decreased, and the product is more deeply colored if the solution of ammonium salt is not cooled before the addition of sodium hydroxide.
3. A series of such runs is made with the ammonium chloride recovered from the trap for each successive run. Of the total N^{15} , 99% is accounted for in the nicotinic acid, the ammonium chloride finally recovered, and the mother liquors by Kjeldahl digestion.
4. Apparently less catalyst would have sufficed.
5. 2-Piperidone-3,4,5,6- H^2-N^{15} is converted into proline-3,4,5- H^2-N^{15} by the method of Heymons⁵ with a few modifications.
6. Care is taken to maintain anhydrous conditions throughout the reaction and distillation.
7. The best yield of copper proline dihydrate was 11.7 g. (32%) from 22.4 g. of 2-piperidone.

¹H. von Pechmann, *Ann.*, 264, 261 (1891).

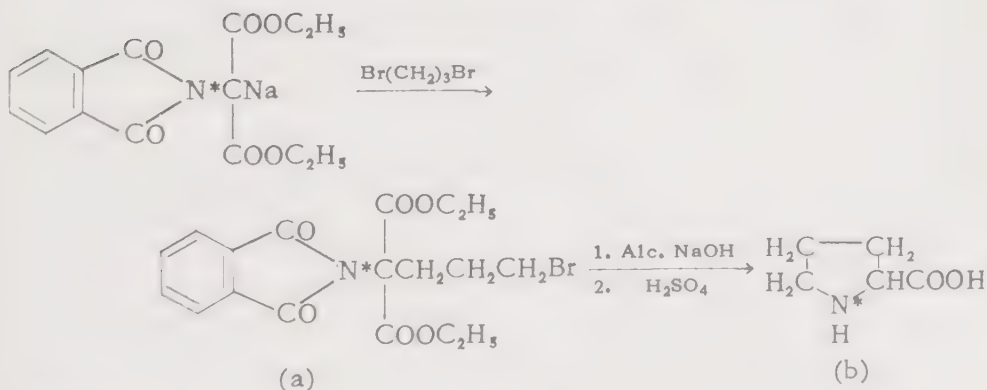
²H. von Pechmann and W. Welsh, *Ber.*, 17, 2384 (1884).

³H. von Pechmann and O. Baltzer, *ibid.*, 24, 3144 (1891).

⁴D. Rittenberg and R. Schoenheimer, *J. Biol. Chem.*, 111, 169 (1935).

⁵A. Heymons, *Ber.*, 66, 846 (1935).

PROLINE- N^{15}
(2-Pyrrolidinecarboxylic- N^{15} Acid)



D. Shemin, *J. Biol. Chem.*, 162, 304 (1946).

A. Procedure

Proline- N^{15} is prepared by adaptation of the following procedure described by Sørensen.¹

(a) *Ethyl α -(3-Bromopropyl)-1,3-dioxo-2-isoindolinemalonate- N^{15}* , [*Ethyl (3-Bromopropyl)phthalimidomalonate- N^{15}*]. To 0.2 mole of ethyl 1,3-dioxo- α -sodium-2-isoindolinemalonate (Note 1), in a flask equipped with a reflux condenser, protected by a drying tube, and a mercury-sealed stirrer, is added 500 g. (0.25 mole) of 1,3-dibromopropane. The mixture is heated in an oil-bath at 150–160°, with stirring, until it is no longer basic. The reaction mixture is cooled and treated with water, and the excess dibromopropane is removed by steam distillation. After cooling the mixture, the water layer is separated from the oily ethyl (3-bromopropyl)phthalimidomalonate (Note 2). According to Sørensen,² the yield is about 80%.

(b) *Proline- N^{15}* , (*2-Pyrrolidinecarboxylic- N^{15} Acid*). Without purification, the above ethyl (3-bromopropyl)phthalimidomalonate, dissolved in 250 ml. of absolute alcohol in a flask equipped with a reflux condenser, is treated with 10 g. of powdered sodium hydroxide (Note 2). After the mixture is warmed on a water-bath for 2 hours, the sodium hydroxide largely dissolves, and a crystalline precipitate of salt appears. To the mixture is added 20 g. more sodium hydroxide, and after heating 2 hours longer on a water-bath, an additional 20 g. of base is added. Then, after 4 hours more of heating, the reaction mixture solidifies to a gel-like mass. After cooling, it is dissolved in 200 ml. of water, and the alcohol is evaporated. The residue is dissolved in 0.5 liter of water, 200 ml. of concentrated hydrochloric acid is added, and the solution is evaporated (Note 3). The sodium chloride and the phthalic acid are largely eliminated by dissolving the product in strong hydrochloric acid. The filtrate is partially evaporated, and the residual phthalic acid is extracted with ether. The aqueous solution is then evaporated on a water-bath to a syrupy mass which is stored over sulfuric acid in a desiccator overnight. The amino acid hydrochloride is then dissolved in absolute alcohol, leaving a small residue of sodium chloride. The alcoholic solution is diluted with water, the alcohol is distilled off, and chloride is removed with silver carbonate. The resulting clear filtrate is evaporated to a small volume *in vacuo* and finally concentrated to a syrup in a dish on a water-bath. After it is stored overnight in a desiccator with sulfuric acid, the solidified mass is pulverized and triturated with 100 ml. of 93% alcohol. After a few hours most of the proline is dissolved, leaving the impurities as a residue. The solution is filtered, and the residue is extracted with two 50-ml. portions and one 100-ml. portion of alcohol. Alcohol is distilled from the combined alcoholic solution, and the residue is dissolved in water. This solution is warmed with copper carbonate and filtered, and, after partial evaporation, the copper salt of proline crystallizes in pure form. By further evaporation of the solution more pure copper salt is obtained, making the yield 21.1 g. (Note 4). The total yield of the copper salt (Note 5), including 1.8 g. from the

mother liquor, is 22.9 g. (87%). The free amino acid is obtained from the copper salt as described in Note 4.

B. Notes

1. Ethyl phthalimidomalonate- N^{15} is prepared by a suitable adaptation of the procedure of Sørensen² as described by Osterberg.³ The sodium derivative is prepared by the method of Sørensen⁴ or by an improved procedure described by Dunn.⁵ See N^{15} -ornithine, Method II.

2. This is a little more than the amount required to neutralize the hydrogen bromide resulting from the ring closure.

3. The residual mushy mass is a mixture of the hydrochlorides of proline and glycine, together with phthalic acid and a large amount of sodium chloride.

4. Additional proline copper salt was obtained from the mother liquor. The solution was acidified with sulfuric acid and treated with hydrogen sulfide to remove copper. Sulfate was then precipitated by the addition of barium hydroxide, and the solution was filtered and evaporated to dryness. The residue, which partially crystallized upon standing, was extracted with alcohol. The filtrate was diluted with water and treated with copper carbonate as before. In this manner an additional 1.8 g. of proline copper salt was obtained.

5. The molecular formula of the copper salt is $(C_5H_8O_2N)_2Cu \cdot 2H_2O$.

¹S. P. L. Sørensen and A. C. Andersen, *Z. physiol. Chem.*, **56**, 236 (1908).

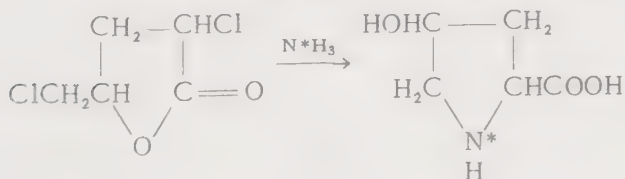
²S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, **6**, 1 (1903); *Chem. Zentr.* **II**, 1903, 33.

³*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 271.

⁴S. P. L. Sørensen, *Z. physiol. Chem.*, **44**, 454 (1905).

⁵M. S. Dunn and B. W. Smart, *J. Biol. Chem.*, **89**, 41 (1930).

4-HYDROXYPROLINE- N^{15} (4-Hydroxy-2-pyrrolidinecarboxylic- N^{15} Acid)



M. R. Stetten, *J. Biol. Chem.*, **181**, 34 (1949).

Procedure

2-Carboxy-5-chloro-4-valerolactone, prepared from epichlorohydrin and sodiummalonic ester according to Traube and Lehmann,¹ is chlorin-

ated and converted to 2,5-dichloro-4-valerolactone by the method of Leuchs.²

Ammonia-N¹⁵, from 17.2 g. of ammonium-N¹⁵ nitrate, is aerated into an ice-cold aqueous-alcoholic solution of 8.5 g. of 2,5-dichloro-4-valerolactone. Reaction of the dichlorolactone with ammonia-N¹⁵ proceeds at room temperature for 10 days; after which time, the remaining free ammonia-N¹⁵ is recovered by passing a stream of air over the heated solution and into an acid trap. To recover the ammonia-N¹⁵ from the ammonium-N¹⁵ salt of 4-hydroxyproline-N¹⁵, the solution is cooled, made alkaline with barium hydroxide, and once again heated while the ammonia-N¹⁵ is aerated into acid. Barium is removed as the sulfate, and chloride ions are also quantitatively removed, as silver chloride, from the aqueous alcoholic solution of 4-hydroxyproline-N¹⁵. The solution is decolorized with carbon, and the filtrate is boiled for several hours with copper oxide. The filtered solution is evaporated to a small volume, and successive crops of copper 4-hydroxyproline-N¹⁵ crystals are obtained upon repeated refrigeration and evaporation.

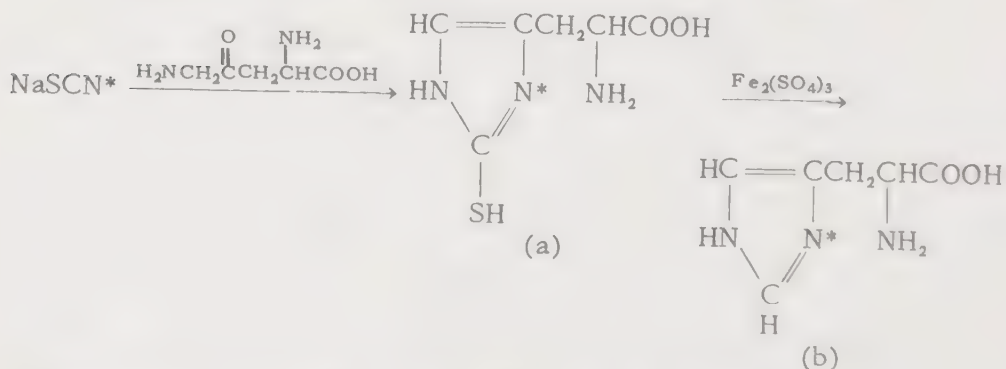
This method of fractionation has been shown² to separate the DL compound, containing the naturally occurring hydroxy-L-proline, from the DL-allo isomer. The first two crops, consisting of 0.965 g. of copper salt, are well formed, dark blue crystals, typical of the less soluble copper hydroxy-DL-proline-N¹⁵ tetrahydrate. The hydrated copper salt, 0.802 g., is dried for 6 hours at 110° over phosphorus pentoxide *in vacuo*. The loss in weight is 0.146 g. or 18.2%. The calculated water content of Cu(C₅H₈O₃N)₂ · 4H₂O is 18.2%. The copper salt is decomposed with hydrogen sulfide, and the 4-hydroxyproline-N¹⁵ is recrystallized from water-alcohol.

¹W. Traube and E. Lehmann, Ber., 34, 1971 (1901).

²H. Leuchs, M. Guia and J. F. Brewster, Ber. 45, 1960 (1912).

L-HISTIDINE-3(or 1)-N¹⁵

[L-α-Amino-4(or 5)-imidazolepropionic-3(or 1)-N¹⁵ Acid]



A. Procedure

(a) *L*-2-Mercaptohistidine-3(or 1)-N¹⁵, (*L*- α -Amino-2-mercapto-4(or 5)-imidazolepropionic-3(or 1)-N¹⁵ Acid). 2-Mercaptohistidine-3(or 1)-N¹⁵ is prepared from γ -ketoornithine^{1,2} (2,5-diamino-4-oxovaleric acid) and sodium thiocyanate-N¹⁵ according to the procedure of Ashley and Harington.² To 21.0 g. of *L*- γ -ketoornithine dihydrochloride dissolved in a little water is added 8.0 g. of sodium thiocyanate-N¹⁵, and the mixture is heated on the steam-bath for 1 hour. Upon treatment of the solution with saturated aqueous sodium acetate until it is no longer acid to Congo red, crystallization of the amino acid begins almost immediately and is complete after several hours at 0°. The yield of *L*-2-mercaptohistidine-3(or 1)-N¹⁵ is 5.94 g., 32%, (Note 1). The product crystallizes from water (Note 2) in colorless plates which darken at 290° but are not melted at 310° (Note 3).

(b) *L*-Histidine-3(or 1)-N¹⁵, [*L*- α -Amino-4(or 5)-imidazolepropionic-3(or 1)-N¹⁵ Acid] (Note 4). The 2-mercaptohistidine, 5.94 g., is heated on a steam-bath with a solution of 80 g. of ferric sulfate in 400 ml. of water for 1 hour. The solution is heated to boiling and treated with barium hydroxide in slight excess. The precipitate is collected, suspended in boiling water and treated with barium hydroxide in slight excess. The precipitate is again collected, suspended in boiling water and filtered off. The combined filtrates are heated to boiling and made faintly acid to Congo red with dilute sulfuric acid. After removal of barium sulfate, the solution is concentrated under vacuum and is treated with flavianic acid. After the solution is kept overnight at room temperature and a few hours at 0°, the crystalline precipitate is collected (Note 5).

The *L*-histidine diflavianate is dissolved in warm dilute sulfuric acid, and the flavianic acid is removed by extraction with butyl alcohol. The aqueous layer is freed of sulfate ions with the equivalent amount of barium hydroxide, and the filtered solution is evaporated to dryness under vacuum. The crystalline residue is dissolved in hot water, and alcohol is added until a permanent turbidity is produced. On being kept overnight at 0°, the solution deposits colorless plates (Note 6). The yield of N¹⁵-*L*-histidine, obtained by adaptation of the above procedure, is 2.64 g. (52%). The product is purified as the monohydrochloride, C₆H₉N₃O₂ · HCl · H₂O.

B. Notes

1. An additional small amount of product may be obtained² by dilution of the mother liquor and precipitation of the amino acid as a mercuric sulfate complex, using a solution of 10% mercuric sulfate in 5% sulfuric acid. The first sticky portion of the precipitate is rejected; the remainder is collected, washed with water and decomposed with hydrogen sulfide. Sulfuric acid is removed from the filtrate as barium sulfate.

Upon concentration of the filtrate under vacuum to a small volume the amino acid crystallizes.

2. L-2-Mercaptohistidine, readily soluble in hot water, is sparingly soluble at room temperature; it displays a marked tendency to form saturated solutions. It is soluble in alcohol and other organic solvents.

3. Although feebly basic, 2-mercaptohistidine forms a well-defined dihydrochloride when its solution in concentrated hydrochloric acid is evaporated in a desiccator. The salt forms in large colorless prisms, m.p. $197-199^{\circ}$ (dec.), which are very soluble in water but sparingly soluble in alcohol.

4. The experimental details are from the work of Ashley and Harington.²

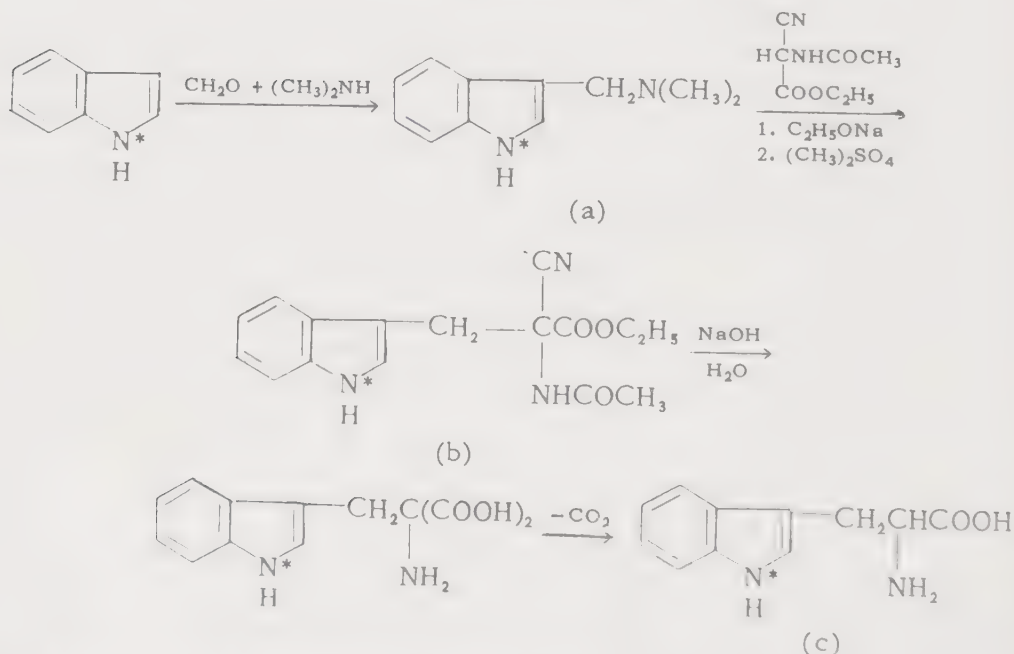
5. The yield of histidine diflavianate is about 83% (based on 2-mercaptohistidine). It crystallizes from a 3% solution of flavianic acid in yellow needles, m.p. $241-242^{\circ}$ (dec.).

6. Ashley and Harington² give the melting point of L-histidine, prepared in this manner, as 275° with decomposition (uncor.).

¹C. Tesar and D. Rittenberg, J. Biol. Chem., 170, 37 (1947).

²J. N. Ashley and C. R. Harington, J. Chem. Soc., 1930, 2586.

TRYPTOPHAN-1-N¹⁵
(α -Amino-3-indolepropionic-1-N¹⁵ Acid)



R. W. Schayer, J. Biol. Chem., 187, 777 (1950).

A. Procedure

Tryptophan-1- N^{15} is prepared by suitable adaptation of the following procedures.

(a) *3-(Dimethylaminomethyl)indole-1- N^{15} , (N_1^{15} -Gramine).* According to Snyder's¹ modification of the procedure of Kuhn,² 140 g. (2.33 moles) of glacial acetic acid is added to 135 g. of 35% dimethylamine (equivalent to 1.05 moles), which is cooled to 5°. With the mixture cooled to 5°, 76 g. of 40% formalin (equivalent to 1.0 mole of formaldehyde), also cooled to 5°, is added. This mixture is agitated mildly and poured into a flask containing 117.2 g. (1 mole) of indole, followed by intermittent shaking of the mixture until the indole dissolves (Note 1). After standing 10 hours, the mixture is added slowly to a stirred solution of 140 g. of sodium hydroxide in 1 liter of water. The resulting suspension is cooled in an ice-bath for 2 hours. The product is collected, pressed dry on the filter, washed with three 100-ml. portions of water and dried at 50° overnight; m.p. 127–128° (Note 2).

(b) *Ethyl α -Acetamido- α -cyano-3-indolepropionate-1- N^{15} .* According to the procedure of Albertson and Tullar,³ the condensation of N_1^{15} -gramine and ethyl acetamidocyanoacetate⁴ is effected under the same conditions employed for acetamidomalonic ester^{5,6} (Note 3). To a solution of 2.76 g. (0.12 mole) of sodium in 250 ml. of absolute ethanol is added 0.12 mole of ethyl acetamidocyanoacetate and 17.4 g. (0.12 mole) of gramine. To this solution, with stirring and cooling, is added 25.2 g. (0.20 mole) of methyl sulfate. The solution, protected from moisture, is stored at room temperature for 4 hours and then poured into water. After the resulting suspension is chilled in ice, the product is collected and dried. The yield of ethyl α -acetamido- α -cyano-3-indolepropionate, m.p. 196–198°, is 98%.

(c) *Tryptophan-1- N^{15} , (α -Amino-3-indolepropionic-1- N^{15} Acid).* Tryptophan-1- N^{15} is obtained by alkaline hydrolysis³ of ethyl α -acetamido- α -cyano-3-indolepropionate-1- N^{15} , followed by decarboxylation, in a manner similar to that described by Snyder⁷ for ethyl α -acetamido- α -carbethoxy-3-indolepropionate. A mixture of 0.1 mole of ethyl α -acetamido- α -cyano-3-indolepropionate and 0.5 mole of sodium hydroxide in 200 ml. of water is refluxed for 24 hours, cooled, and neutralized with concentrated hydrochloric acid to pH 7. The neutral solution is then refluxed for an additional 2.5 hours to effect decarboxylation of the intermediate α -amino- α -carboxy-3-indolepropionic acid. After the solution is stored in the refrigerator for 12 hours, the solid is collected and dissolved in 2.5 *N* sodium hydroxide solution. The basic solution is treated with carbon, diluted with one-half its volume of 95% ethanol, warmed to 70°, acidified with 7.5 ml. of glacial acetic acid and allowed to cool slowly. The

product is collected, washed with water, ethanol and ether, and recrystallized from 33% ethanol (Notes 4 and 5).

B. Notes

1. The temperature of the mixture rose to 56° during this time.

2. Albertson⁵ prepared gramine by this method in 90–93% yield; m.p. $128\text{--}130^{\circ}$. A purer product, m.p. 134° , was obtained by recrystallization from acetone, but losses were excessive.

3. Snyder⁷ condensed gramine methiodide¹ with ethyl sodioacetamidomalonate in dry dioxane to obtain ethyl α -acetamido- α -carbethoxy-3-indolepropionate in yields ranging from 63–70%. Albertson⁵ found it unnecessary to use the quaternary salt in the condensation. When ethyl iodide was added slowly to a warm solution of gramine and ethyl sodioacetamidomalonate in absolute ethanol, the desired product was isolated in 73% yield. Recovery of 16% of the gramine made the yield, based on the consumed Mannich base, actually 86%. The reaction in dry ethanol was complete in 7–8 hours compared to 36 hours in the dioxane procedure. Alkaline degradation of the ester to tryptophan in 81% yield reported by Snyder⁷ was superior to simultaneous hydrolysis and decarboxylation in 2 N sulfuric acid; yield, 61%.⁵

Albertson⁵ substituted methyl sulfate for ethyl iodide in the condensation, and a nearly quantitative conversion of gramine to ethyl α -acetamido- α -carbethoxy-3-indolepropionate was realized when two moles of methyl sulfate, per mole of gramine, were used in the reaction. When two moles of methyl sulfate were used, the demands of both the volatile base, *N,N*-dimethylethylamine, and the Mannich base were satisfied, and the latter then reacted completely.

4. Since no details of the alkaline hydrolysis were given by Albertson,³ the procedure was taken, in part, from the work of Snyder.⁷

5. Albertson³ obtained a 71% yield of tryptophan, m.p. $288\text{--}290^{\circ}$ (cor.), based on crude indole.

¹H. R. Snyder, C. W. Smith and J. M. Stewart, *J. Am. Chem. Soc.*, **66**, 200 (1944).

²H. Kuhn and O. Stein, *Ber.*, **70**, 567 (1937).

³N. F. Albertson and B. F. Tullar, *J. Am. Chem. Soc.*, **67**, 502 (1945).

⁴V. Cerchez and Mme. Dumitresco-Colesiu, *Compt. rend.*, **194**, 1954 (1932).

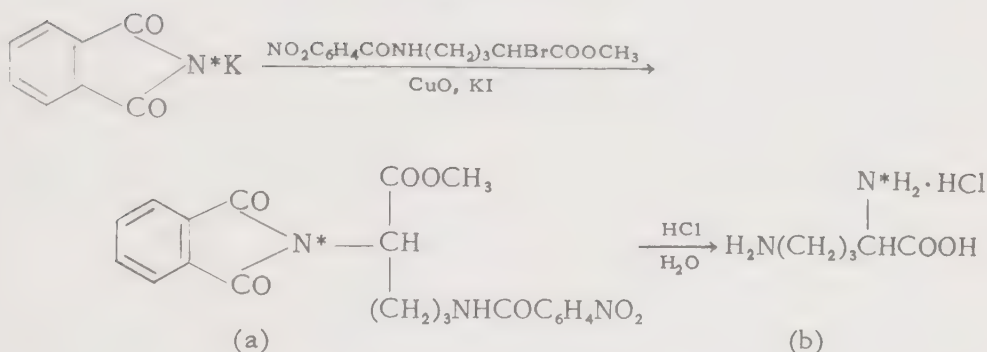
⁵N. F. Albertson, S. Archer and C. M. Suter, *J. Am. Chem. Soc.*, **67**, 36 (1945).

⁶N. F. Albertson and S. Archer, *ibid.*, **67**, 308 (1945).

⁷H. R. Snyder and C. W. Smith, *ibid.*, **66**, 350 (1944).

DL-ORNITHINE- N^2 - N^{15} HYDROCHLORIDE

METHOD I



C. H. W. Hirs and D. Rittenberg, J. Biol. Chem., 186, 439 (1950).

A. Procedure (Note 1)

(a) *Methyl 2-(Phthalimido- N^{15})-5-(3-nitrobenzamido)valerate*. Methyl 2-bromo-5-(3-nitrobenzamido)valerate (Note 2) is heated with potassium phthalimide- N^{15} , in the presence of cupric oxide and potassium iodide, in a metal-bath at 200° for 3.5 hours. The product is extracted into alcohol, filtered and evaporated to dryness.

(b) *DL-Ornithine- N^2 - N^{15} Hydrochloride*, (*DL-2,5-Diaminovaleric-2- N^{15} Acid Hydrochloride*). The crude valeric ester, above, is heated under reflux with 20% hydrochloric acid for 18 hours. The *m*-nitrobenzoic and phthalic acids are removed by extraction with ether, and the aqueous solution is evaporated to dryness. The residue is dissolved in water, treated with carbon and again evaporated to dryness. The solid is dissolved in 95% alcohol, and the monohydrochloride of ornithine- N^2 - N^{15} is precipitated by the addition of pyridine. The product is further purified by dissolution in water, followed by precipitation with alcohol.

Resolution of DL-Ornithine- N^2 - N^{15} . DL-Ornithine- N^2 - N^{15} is resolved by the method of Sørensen¹ (Note 3). For resolution a mixture of 6.00 g. (35.6 mmoles) of normal DL-ornithine monohydrochloride and 0.845 g. (5.0 mmoles) of DL-ornithine- N^2 - N^{15} hydrochloride is benzoylated (Note 4). Of the resulting DL-ornithuric- N^2 - N^{15} acid (2,5-dibenzamidovaleric-2- N^{15} acid), 5 g. (14.7 mmoles) is used in the following procedure, described by Sørensen.¹

(c) *D-Ornithine- N^2 - N^{15}* . A mixture of 34 g. (0.1 mole) of DL-ornithuric acid, 46.6 g. (0.1 mole) of brucine hydrate and 3 l. of water is heated with stirring for 2 hours. At this time, almost all the solid has dissolved, and the solution is warmed for an additional 4 hours. By this time

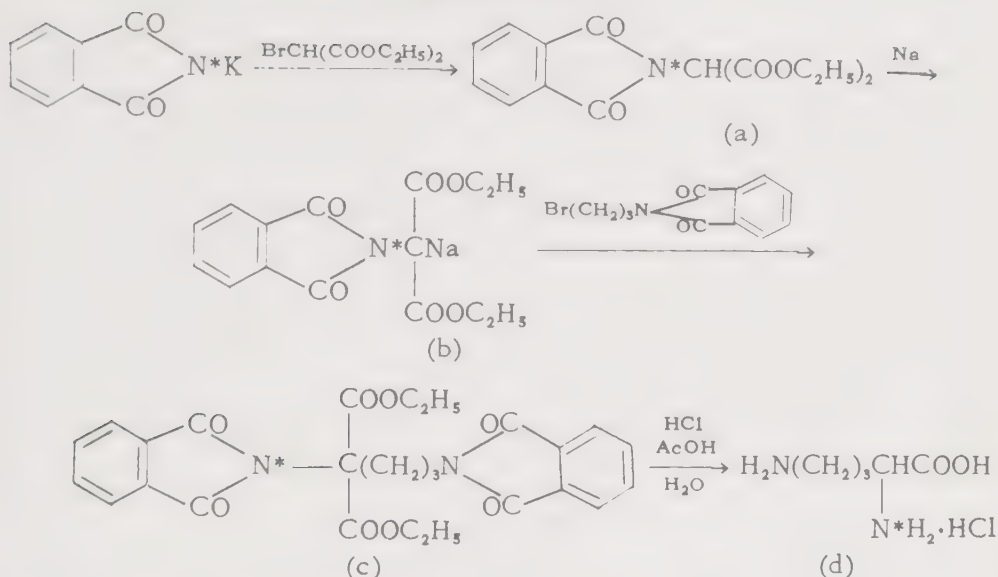
crystallization has started, and the mixture is kept for about 12 hours with frequent agitation. The slightly colored crystalline brucine D-ornithurate is collected and washed with 3 portions of cold water; after drying in air, it weighs 28.5 g. The product is dissolved in a total of 5 l. of hot water, filtered and crystallized overnight. After a second recrystallization, 13.3 g. of the brucine D-salt is obtained. By partial evaporation of each mother liquor, a total of 11.8 g. (34.7%) of the original DL-mixture is obtained in the D-ornithuric acid fraction. Brucine is eliminated by warming each fraction with water and slightly more than 1 equivalent of sodium hydroxide. After the mixture is cooled, the precipitate of brucine is collected and washed with cold water.

The alkaline filtrate is acidified with hydrochloric acid and left overnight. The precipitate of ornithuric acid is collected and washed several times with cold water and then with warm water. To eliminate traces of brucine, the product is redissolved in base and again precipitated. After hydrolysis of the D-ornithuric acid, D-ornithine is isolated in the manner described for the DL-mixture (b).

(d) *L*-Ornithine- N^2 - N^{15} . By further concentration of the mother liquors from the brucine D-ornithurate, the brucine salt of *L*-ornithuric acid, contaminated with some of the racemate, is obtained (see Note 3). Freed of brucine, as described above, this fraction amounts to 51% of the DL-mixture. A mixture of 21.6 g. of impure *L*-ornithuric acid and 19.5 g. of cinchonine is powdered in a mortar and suspended in 0.5 l. of water. With stirring, 4 l. of boiling water is added; most of the solid goes into solution. The mixture is warmed 5–6 hours in a boiling water-bath, with stirring, and then filtered. Upon cooling, the filtrate becomes milky, and with agitation and further cooling a crystalline product is obtained. After remaining overnight, the crystals are collected, washed with cold water 3 times and dried. The yield of cinchonine *L*-ornithurate is 20.2 g. A total of 39.0 g. of *L*-ornithuric acid which was treated in the above manner furnished 34.2 g. of the cinchonine salt. In order to recrystallize this material (Note 6), it is suspended in 0.5 l. of cold water; then, with vigorous agitation, boiling water is added until the salt is nearly all dissolved. The hot solution is filtered, and the remaining solid is treated in like manner. The total solution (5–6 liters, Note 7) is evaporated on a water-bath until a film of crystals appears on the surface. The solution is cooled with constant agitation, and the crystallization is completed overnight. The crystalline cinchonine *L*-ornithurate, which is collected, washed 3 times with cold water and dried, weighs 16 g. From the mother liquors and washings an additional 11.2 g. of impure *L*-salt is obtained.

L-Ornithine is prepared from the purified cinchonine *L*-ornithine in the manner described above for the D-isomer (Note 8).

METHOD II



M. R. Stetten, J. Biol. Chem., 189, 499 (1951).

A. Procedure

(a) *Ethyl Phthalimidomalonate- N^{15}* (Note 9). Equimolar quantities of potassium phthalimide- N^{15} and ethyl bromomalonate are stirred together. If spontaneous reaction does not start within one-half hour (Note 10), it is necessary to initiate the reaction in an oil-bath at $110\text{--}120^\circ$. When the temperature of the liquid mixture begins to drop, the mixture is heated in an oil-bath at 110° for 1 hour. When cold, the mixture is ground up with water and filtered. The precipitate is reground with water, collected and washed well with water (Note 11). Without drying, the solid is extracted with boiling benzene which is filtered, after cooling, to remove insoluble potassium phthalimide and bromide. The filtrate consists of an aqueous and a benzene layer which are separated, and the benzene layer is dried over calcium chloride. The benzene is removed by distillation on a water-bath, and the residue solidifies on cooling. The crystalline mass is then ground with small portions of ether, filtered, and washed with ether until the product is white. The yield (Note 12) of ethyl phthalimidomalonate- N^{15} , m.p. $73\text{--}74^\circ$, is 67–71%.

(b) *Ethyl Phthalimidosodiummalonate- N^{15}* (Note 13). In a 2-l. 3-necked flask, equipped with condenser, dropping funnel and mechanical stirrer, are placed 400 ml. of dry toluene and 163.5 g. of ethyl phthalimidomalonate. After heating the mixture to dissolve the ester, 12.3 g. of purified sodium (Note 14) is added. The mixture is refluxed for 1.5 hours, cooled and filtered. The product is washed with 200 ml. of toluene and dried at 105° for 24 hours. The yield of light-yellow product,

which decomposes into a dark-brown oil at 280° , is 148 g. (85%) (Note 15).

(c) *Ethyl 2-Ethoxycarbonyl-2,5-diphtalimidovalerate-2-N¹⁵*. Using an adaptation of the procedure of Fink² (see ethyl 2-ethoxycarbonyl-2,6-diphtalimidohexanoate-6-N¹⁵), equimolar quantities of ethyl phthalimido-sodiummalonate and *N*-(3-bromopropyl)phthalimide are stirred and heated for 5 hours at 150 – 160° . The reaction mixture is cooled and extracted with three portions of hot absolute alcohol. The extracts are filtered while hot and concentrated to a thick syrup *in vacuo*.

(d) *DL-Ornithine-N²-N¹⁵ Hydrochloride*, (*DL-2,5-Diaminovaleric-2-N¹⁵ Acid Hydrochloride*). The diphtalimido ester is simultaneously hydrolyzed and decarboxylated as described under ornithine-N⁵-N¹⁵ (Note 16). The over-all yield of *DL-ornithine-N²-N¹⁵* monohydrochloride, from 22.8 g. of potassium phthalimide-N¹⁵, is 3.34 g. (16%).

B. Notes

1. *DL-Ornithine-N²-N¹⁵* was prepared according to a modification of the procedure of Clutton.³

2. 2-Bromo-5-(3-nitrobenzamido)valeric acid, m.p. 125° , was prepared in 65% yield by Clutton,³ from 5-(3-nitrobenzamido)valeric acid, according to the procedure of Fischer.⁴

3. In this procedure, success was regularly attained only when crystallization of the L-salt of brucine was initiated by seeding with brucine L-ornithurate monohydrate, m.p. 136° , prepared from pure L-ornithuric acid.

4. *DL-Ornithine* may be benzoylated by modification of the procedure of Ingersoll and Babcock⁵ for the preparation of hippuric acid.

5. Brucine D-ornithurate is slightly soluble in hot water, soluble in cold and very soluble in hot alcohol, and very slightly soluble in ether. Recrystallized from water and air-dried, the salt contains one molecule of water of crystallization, which it loses in vacuum at 70° .

6. When warmed with water, in a boiling water-bath, the cinchonine salt fused and was dissolved with difficulty. The same result was had when boiling water was poured on the salt.

7. Upon cooling the large volume, an insignificant amount of salt crystallized.

8. From 5 g. of *DL-ornithuric-N²-N¹⁵* acid, Hirs and Rittenberg obtained 0.664 g. (54%) of *L-ornithine-N²-N¹⁵* monohydrochloride.

9. Ethyl phthalimidomalonate-N¹⁵ may be prepared by incorporating the use of potassium phthalimide-N¹⁵ into Osterberg's adaptation⁵ of Sörensen's procedure,⁶ as follows.

10. When freshly prepared ethyl bromomalonate is used, the temperature of the mixture may rise spontaneously to 140° ; no heat should be applied until the temperature falls again.

11. The solid material consists of potassium bromide, phthalimide and the ethyl phthalimidomalonate.

12. A small amount of product may be recovered by distilling the ether from the filtrate and washing the residue with a smaller amount of ether.

13. Sodium phthalimidomalononic ester is prepared by adaptation of the procedure of Dunn and Smart,⁷ which follows.

14. Freshly cut sodium was purified by heating and shaking it in toluene. The resulting shiny balls were weighed in toluene and then transferred to the reaction mixture.

15. Sodium phthalimidomalononic ester gradually hydrolyses in cold water. It is very soluble in methyl alcohol, less so in ethyl and isopropyl alcohol, and is practically insoluble in benzene and toluene.

16. Also see the procedure of Fink² in the synthesis of DL-lysine- N^6 - N^{15} .

¹S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, 6, 209 (1906).

²R. M. Fink, T. Enns, C. P. Kimball, H. E. Silberstein, W. F. Bale, S. C. Madden and G. H. Whipple, *J. Exptl. Med.*, 80, 457 (1944).

³R. F. Clutton, R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, 132, 229 (1940).

⁴E. Fischer and G. Zemplén, *Ber.*, 42, 2989 (1909).

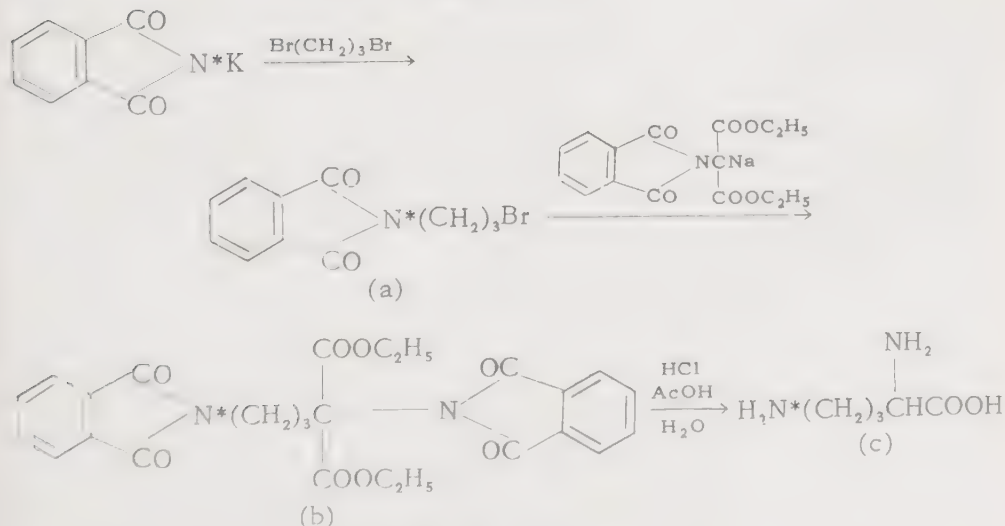
⁵*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 271.

⁶S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, 6, 1 (1903).

⁷M. S. Dunn and B. W. Smart, *J. Biol. Chem.*, 89, 41 (1930).

ORNITHINE- N^5 - N^{15} (2,5-Diaminovaleric-5- N^{15} Acid)

METHOD I



M. R. Stetten, *J. Biol. Chem.*, 189, 499 (1951).

A. Procedure

(a) *N*-(3-Bromopropyl)phthalimide- N^{15} . Potassium phthalimide- N^{15} , 20 g., is reacted with 100 g. of trimethylene bromide according to the method of Gabriel and Weiner,¹ which follows.

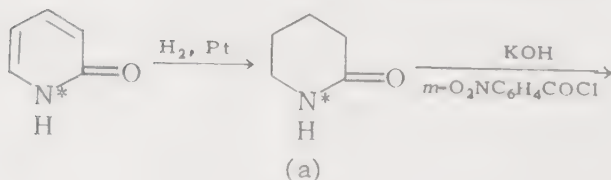
Potassium phthalimide, 70 g., and 210 g. of trimethylene bromide in a 1-l. flask are heated under reflux in an oil-bath at about 170°. The reaction is apparently complete in 10–15 minutes (Note 1), but heating at 170° is continued for 3/4 hour. The viscous reaction product is cooled to about 100°, and the unreacted trimethylene bromide is removed by steam distillation. The residual oil is separated, dried and dissolved in 80 ml. of boiling alcohol. Upon cooling the solution, 75 g. of a crystalline product is obtained, which is best purified by extraction with ether in a Soxhlet apparatus (Note 2). The ether is distilled to obtain 63 g. of *N*-(3-bromopropyl)phthalimide, which still contains a small amount of the diphtalimido compound. The product is recrystallized from ligroin in long needles, m.p. 72–73° (Note 3).

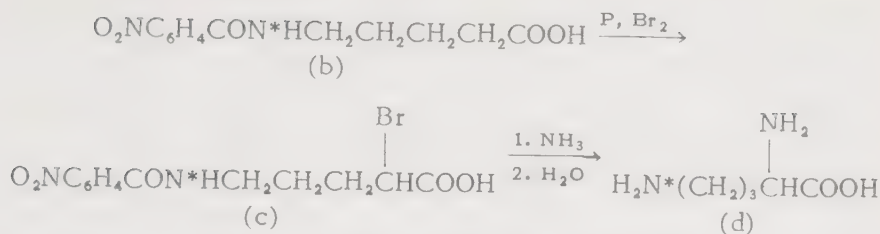
In the isotopic synthesis, 18.8 g. of recrystallized *N*-(3-bromopropyl)-phthalimide- N^{15} is obtained.

(b) *Ethyl 2-Ethoxycarbonyl-2,5-diphtalimidovalerate-2-N¹⁵*. The 18.8 g. of *N*-(3-bromopropyl)phthalimide- N^{15} is condensed with the sodium compound (Note 4) prepared from 22.4 g. of phthalimidomalonic ester.² The two reagents are heated at 150° to 160° for two hours with stirring. The product is extracted with three 100-ml. portions of hot alcohol, filtered while hot and concentrated to a thick syrup *in vacuo*.

(c) *Ornithine-N⁵-N¹⁵*, (*2,5-Diaminovaleric-5-N¹⁵ Acid*). The syrupy diphtalimido compound is heated under reflux for 2.5 hours with 200 ml. each of concentrated hydrochloric acid, glacial acetic acid and water.³ A fraction boiling below 108° is distilled off with the aid of a fractionating column, and heating under reflux is continued for 12 hours. After cooling the solution, phthalic acid is collected, and the filtrate is evaporated to dryness *in vacuo*. The residue is dissolved in a minimum of cold water, pyridine is added, and the DL-ornithine- N^5 - N^{15} monohydrochloride is precipitated by the addition of alcohol. The crude product is recrystallized from water-alcohol; yield, 5.43 g. (30% based on potassium phthalimide- N^{15}).

METHOD II





C. H. W. Hirs and D. Rittenberg, J. Biol. Chem., 186, 429 (1950).

A. Procedure

(a) *2-Piperidone-N¹⁵* (Note 5). In a hydrogenating vessel surrounded by a steam-jacket are placed 12 g. of 2(1*H*)-pyridone-N¹⁵, 12 ml. of dry acetic acid and 1.2 g. of platinum catalyst.⁴ This mixture, heated to 100°, is shaken with hydrogen at 1 atmosphere until 2 molar equivalents of hydrogen are absorbed. The mixture is cooled, diluted with water and filtered. Acetic acid is distilled from the filtrate in *vacuo*, and the residual 2-piperidone-N¹⁵ is purified by distillation, b.p. 134° (14 mm.)⁵ (Note 6).

(b) *5-(3-Nitrobenzamido-N¹⁵)valeric Acid* (Note 7). The residual 2-piperidone-N¹⁵ is refluxed for 3 hours with 120 ml. of 15% sodium hydroxide solution. The resulting solution is diluted to 500 ml. with water, 25 g. of sodium bicarbonate is added, and, with vigorous stirring, 36 g. of 3-nitrobenzoyl chloride, dissolved in 100 ml. of ether, is added in 4 portions during 2 hours. Evaporation of the ether layer leaves a crystalline residue of 1-(3-nitrobenzoyl)-2-piperidone-N¹⁵. This is dissolved during 30 minutes in 110 ml. of 2% sodium hydroxide solution, heated on a water-bath. This solution is combined with the alkaline solution obtained from the benzoylation and acidified with hydrochloric acid. The precipitated product is collected and washed with cold water and ether (Note 8). The product is redissolved in aqueous sodium carbonate, precipitated with hydrochloric acid, collected, washed with cold water and dried, m.p. 134°. The yield based on 2-pyridone-N¹⁵ is 83.5% (Note 9).

(c) *2-Bromo-5-(3-nitrobenzamido-N¹⁵)valeric Acid*. 5-(3-Nitrobenzamido-N¹⁵)valeric acid is brominated by adaptation of the following procedure of Fischer and Zemlén.⁵ A mixture of 50 g. of 5-(3-nitrobenzamido)valeric acid and 6 g. of red phosphorus is ground in a mortar. The mixture is cooled with a water-bath, and 125 g. of bromine is added dropwise during 12–15 minutes. There is rapid evolution of hydrogen bromide, and the mixture is warmed on a water-bath for not more than 12 minutes; the turbulent reaction then slows, and the dark-brown oil cools. After the addition of 250 ml. of ice-cold water, sulfurous acid is introduced, with cooling, until the oil becomes grey in color. The mixture is then diluted with 500 ml. of water, and an excess of sodium carbonate is added with stirring. The solution is filtered, and acidification of the

filtrate precipitates a viscous, colorless oil that soon crystallizes. The yield of practically pure product is 42 g. (65%). After recrystallization from hot 60% alcohol and drying over phosphorus pentoxide *in vacuo*, the colorless needles sinter at 120° and melt at 125°. The product is quite soluble in acetone, ethyl acetate and hot alcohol and slightly soluble in ether and benzene.

(d) *Ornithine-N⁵-N¹⁵*, (2,5-Diaminovaleric-5-N¹⁵ Acid). In accordance with the procedure of Clutton,⁶ 14 g. of 2-bromo-5-(3-nitrobenzamido-N¹⁵)valeric acid is dissolved in 100 ml. of concentrated ammonium hydroxide, and the solution is saturated at 0° with ammonia gas. After 48 hours, ammonia is again passed through the solution, which is left for an additional 48 hours. Water and excess ammonia are removed under reduced pressure, and the residue is refluxed for 18 hours with 350 ml. of 20% hydrochloric acid. 3-Nitrobenzoic acid is removed by extraction with ether; the aqueous solution is evaporated to dryness, and the residue is redissolved in water, treated with carbon and again concentrated to dryness. The residue is extracted with a total of 100 ml. of 95% ethanol (Note 10), and ornithine-N⁵-N¹⁵ monohydrochloride is precipitated by the addition of 3.5 ml. of pyridine. The yield of crude product is 5.4 g. (100%). It is further purified by precipitation from aqueous solution with 95% alcohol (Note 11).

(e) *Resolution of DL-Ornithine-N⁵-N¹⁵*. D- and L-Ornithine-N⁵-N¹⁵ are prepared according to the procedure of Sørensen;⁷ see D- and L-ornithine-N²-N¹⁵.

B. Notes

1. This is apparent from the disappearance of the potassium phthalimide.

2. The residue of about 6 g. is 1,3-dipthalimidopropane.

3. This compound is very slightly soluble in water, slightly soluble in ligroin but quite soluble in warm alcohol and ether.

4. The method of Sørensen⁸ is cited for preparation of the sodium compound; however, a much improved procedure is described by Dunn and Smart.⁹

5. 2-Pyridone-N¹⁵ was hydrogenated according to the procedure of Stetten¹⁰ as modified by Clutton.⁶

6. Stetten¹⁰ reported an 83% yield.

7. In the procedure of Clutton,⁶ which follows, the 2-pyridone-N¹⁵ was not isolated and purified. The residue left upon removal of the acetic acid was used directly in the next step of the synthesis.

8. Ether removes 3-nitrobenzoic acid.

9. The product may be recrystallized from 60% ethanol.

10. This extraction eliminates most of the ammonium chloride.

11. The overall yield of DL-ornithine- N^5 - N^{15} prepared by this method was 38%.

¹S. Gabriel and J. Weiner, *Ber.*, 21, 2669 (1888).

²S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg*, 6, 6 (1905).

³R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, 127, 310 (1939).

⁴*Organic Syntheses*, Coll. Vol. I, 2nd ed, Wiley, New York, 1941, p. 463.

⁵E. Fischer and G. Zemplén, *Ber.*, 42, 2989 (1909).

⁶R. F. Clutton, R. Schoenheimer and D. Rittenberg, *J. Biol. Chem.*, 132, 227 (1940).

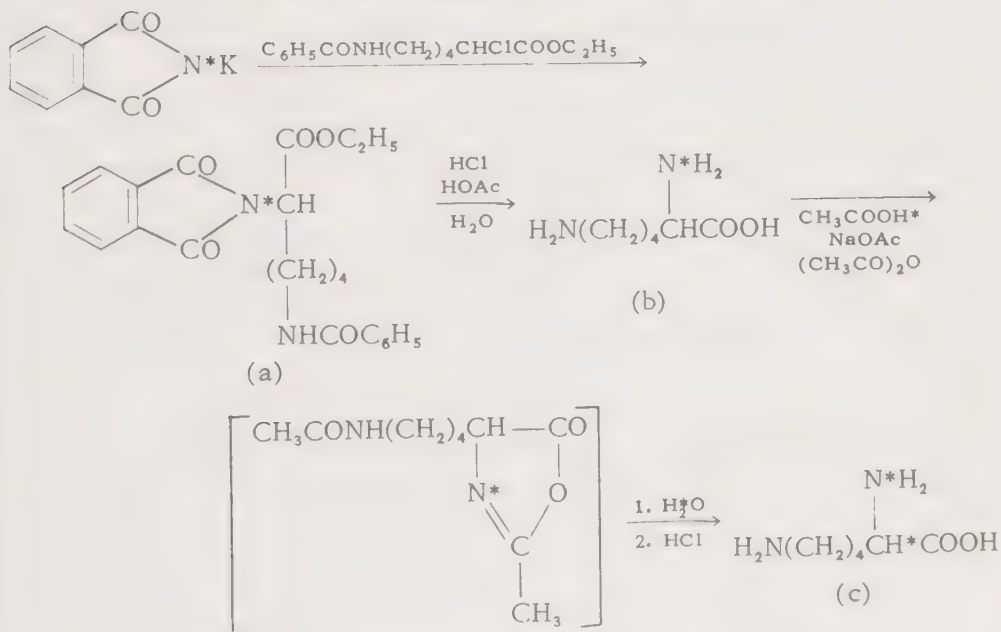
⁷S. P. L. Sørensen, *Compt. rend. trav. lab. Carlsberg* 6, 209 (1906).

⁸S. P. L. Sørensen, *Z. physiol. Chem.*, 44, 448 (1905).

⁹M. S. Dunn and B. W. Smart, *J. Biol. Chem.*, 89, 46 (1930).

¹⁰M. R. Stetten and R. Schoenheimer, *ibid.*, 153, 113 (1944).

LYSINE-2- H^2 - N^2 - N^{15}
(2,6-Diaminohexanoic-2- H^2 -2- N^{15} Acid)



I. Clark and D. Rittenberg, *J. Biol. Chem.*, 189, 521 (1951).

A. Procedure

(a) *Ethyl 6-Benzamido-2-phthalimidohexanoate-2- N^{15}* . According to a modification of the procedure of Weissman¹ (see lysine-3,4,5- H^2 - N^2 - N^{15}), a mixture of ethyl 6-benzamido-2-chlorohexanoate (Note 1), potassium phthalimide- N^{15} , cupric oxide and a small amount of potassium iodide is heated at 185–200°, with stirring, for 4.5 hours. The product is extracted from the mixture with hot absolute alcohol and concentrated to dryness.

(b) *Lysine- N^2 - N^{15} Dihydrochloride*, (*2,6-Diaminohexanoic-2- N^{15} Acid Dihydrochloride*). The above phthalimido ester is hydrolyzed, and the product is isolated according to Weissman¹. The yield of lysine- N^2 - N^{15} dihydrochloride is 80% of theoretical.

(c) *Lysine-2- H^2 - N^2 - N^{15}* , (*2,6-Diaminohexanoic-2- H^2 -2- N^{15} Acid*) (Note 2). To a mixture of 1.8 moles of acetic acid- H^2 and 0.14 mole of fused sodium acetate is added 0.13 mole of lysine- N^2 - N^{15} dihydrochloride. After the mixture is refluxed for 1 hour, 0.36 mole of acetic anhydride is added, and the temperature is maintained at 100° for 2 hours. The reaction mixture is cooled, treated with 0.6 mole of water- H_2^2 and then refluxed for 1 hour. The solvent is removed, 20 per cent hydrochloric acid is added, and the mixture is refluxed for 6 hours to effect hydrolysis of the acetyl groups. The lysine-2- H^2 - N^2 - N^{15} is isolated as described,¹ under lysine-3,4-5- H_3^2 - N^2 - N^{15} .

Resolution of DL-lysine-2- H^2 - N^2 - N^{15} . DL-Lysine-2- H^2 - N^2 - N^{15} is resolved by the carbobenzyloxy-aniline-papain method of Bergmann²⁻⁴ (Note 3). A recent application⁵ of this method to DL-lysine is as follows.

(d) *N^2 , N^6 -Bis(benzyloxycarbonyl)-DL-lysine-2- H^2 - N^2 - N^{15}* . A solution of 0.430 g. of DL-lysine dihydrochloride, dissolved in 2.9 ml. of 2*N* sodium hydroxide, is cooled with an ice-bath and 1.1 ml. of benzyloxycarbonyl chloride and 2.3 ml. of 4 *N* sodium hydroxide are added in four portions, and the mixture is agitated vigorously for 25 minutes, with cooling. After extraction with ether, the aqueous phase is acidified with hydrochloric acid. The product is extracted into ether, concentrated and dried *in vacuo*. The yield of N^2 , N^6 -bis(benzyloxycarbonyl)-DL-lysine, m.p. 100–103°, is 0.733 g.

(e) *N^2 , N^6 -Bis(benzyloxycarbonyl)-L-lysine-2- H^2 - N^2 - N^{15} Anilide*. To a solution of 0.730 g. of N^2 , N^6 -bis(benzyloxycarbonyl)-DL-lysine, dissolved in 1.9 ml. of 1 *N* sodium hydroxide and 1.8 ml. of water, is added 0.46 ml. of aniline, 7.1 ml. of a 0.3% aqueous solution of cysteine hydrochloride, 8.2 ml. of citrate buffer (pH 5.0), 16.4 ml. of water, a solution of 0.125 g. of papain in 2.5 ml. of water, and 2 ml. of citrate buffer. After incubation of the mixture at 40° for 19 hours, the L-anilide is collected, washed once with 1% potassium bicarbonate solution and three times with water. The product, 0.465 g., melts at 121–122° after recrystallization from 50% ethanol (Note 4).

(f) *L-Lysine-2- H^2 - N^2 - N^{15} Dihydrochloride*. The above L-anilide is refluxed with 4.5 ml. of 6 *N* hydrochloric acid for 2 hours. After cooling, the solution is diluted with 30 ml. of water and treated with freshly prepared silver oxide. The silver chloride and excess silver oxide are removed and washed with water. The filtrate is extracted with ether; the clear aqueous phase is concentrated *in vacuo* to 20 ml., acidified with hydrochloric acid and set aside for 30 minutes with occasional shaking. The solution is filtered and evaporated to dryness *in vacuo* over sodium

hydroxide and sulfuric acid. The yield of L-lysine dihydrochloride is quantitative; $[\alpha]_D^{23} +15.95^\circ$ (c, 5.46 in water) (Note 5).

(g) *D*-Lysine-2- H^2 - N^2 - N^{15} Dihydrochloride. The filtrate (without washings) from the L-anilide preparation, above, is incubated with 25 mg. of papain for 5 days at 40° . After removing the precipitate of mixed L- and D-anilides, the filtrate is acidified with hydrochloric acid and extracted with ether. After evaporation of the solution, the remaining 0.243 g. of N^2 , N^6 -bis(benzyloxycarbonyl)-D-lysine is refluxed with 3.0 ml. of 6 *N* hydrochloric acid for 2 hours. The solution is cooled, extracted with ether and evaporated to dryness. The yield of D-lysine dihydrochloride, after recrystallization from ethanol, is 99 mg.; $[\alpha]_D^{23} -13.5^\circ$, (c, 4.47 in water) (Note 6).

B. Notes

1. 6-Benzamido-2-chlorohexanoic acid was synthesized according to Galat⁶ and esterified with absolute alcohol containing 3% of concentrated sulfuric acid.

2. It has been observed⁷⁻⁹ that certain optically active amino acids, i.e., those with a primary α -amino group, undergo racemization when treated with acetic anhydride. du Vigneaud⁹ proposed a mechanism involving the formation and hydrolysis of an intermediate azlactone, with a tautomeric shift of the α -hydrogen atom giving rise to racemization. During this process the α -hydrogen atom should be exchangeable with deuterium of the medium.

3. Another method of resolution is given in the preparation of D- and L-lysine-3,4,5- H^2 - N^2 - N^{15} .

4. The N^2 , N^6 -bis(benzyloxycarbonyl)-L-lysine-2- H^2 - N^2 - N^{15} anilide prepared by Clark and Rittenberg melted at $125-126^\circ$ (uncor.).

5. Berg¹⁰ reports rotations of $+15.63$ to $+16.55^\circ$ for values of *c* from 3.00 to 16.00.

6. This rotation corresponds to approximately 92.5% D- and 7.5% L-lysine.

¹N. Weissman and R. Schoenheimer, *J. Biol. Chem.*, **140**, 786 (1941).

²M. Bergmann and H. Fraenkel-Conrat, *ibid.*, **119**, 707 (1937).

³J. S. Fruton, G. W. Irving, Jr., and M. Bergmann, *ibid.*, **133**, 703 (1940).

⁴O. K. Behrens, D. G. Doherty and M. Bergmann, *ibid.*, **136**, 61 (1940).

⁵H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley, and P. H. Lowy, *ibid.*, **176**, 1383 (1948).

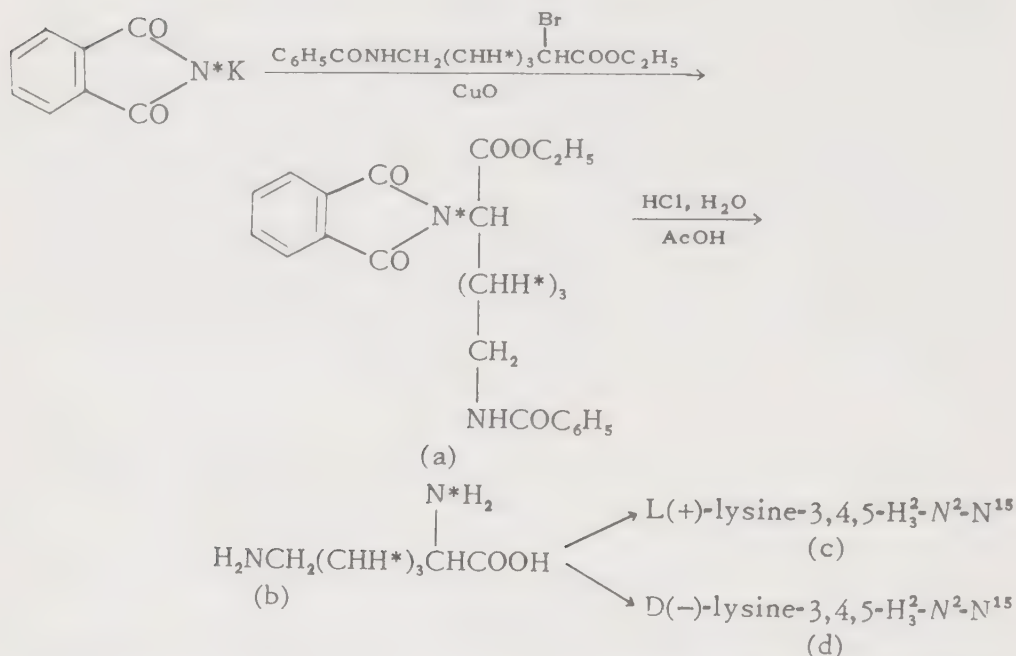
⁶A. Galat, *J. Am. Chem. Soc.*, **69**, 86 (1947).

⁷V. du Vigneaud and R. R. Sealock, *J. Biol. Chem.*, **96**, 511 (1932).

⁸M. Bergmann and L. Zervas, *Biochem. Z.*, **203**, 280 (1928).

⁹V. du Vigneaud and C. E. Meyer, *J. Biol. Chem.*, **99**, 143 (1932-1933).

¹⁰C. P. Berg, *ibid.*, **115**, 9 (1936).

L(+)- and D(-)-LYSINE-3,4,5- H_3^2 - N^2 - N^{15} DIHYDROCHLORIDE

N. Weissman and R. Schoenheimer, J. Biol. Chem., 140, 786 (1941).

A. Procedure

(a) *Ethyl 6-Benzamido-2-phthalimidohexanoate-3,4,5- H_3^2 -2- N^{15}* . To 29.9 g. of ethyl 6-benzamido-2-bromohexanoate-3,4,5- H_3^2 in a 100-ml. tube is added 16.1 g. of potassium phthalimide- N^{15} and 3 g. of cupric oxide. The mixture is heated at 150° with stirring for 4.5 hours and is then extracted with hot absolute alcohol. The solution is filtered, and the solvent is removed, leaving a residue of the crude phthalimido ester.

(b) *DL-Lysine-3,4,5- H_3^2 - N^2 - N^{15} Dihydrochloride, (DL-2,6-Diaminohexanoic-3,4,5- H_3^2 -2- N^{15} Acid Dihydrochloride)*. The crude phthalimido ester is heated under reflux with a mixture of 75 ml. each of glacial acetic acid, concentrated hydrochloric acid and water. After 2.5 hours, ethyl acetate, acetic acid and water are removed by distillation until the vapor temperature is 107° . Heating of the residual mixture under reflux is then continued for 15 hours. Most of the benzoic and phthalic acids are removed by concentrating and filtering the solution. The thick syrup finally obtained is dissolved in 60 ml. of absolute ethanol, and the dihydrochloride is precipitated by addition of ether. The 15.7 g. of crude product is twice recrystallized from alcohol-acetone to obtain 11.9 g. of lysine-3,4,5- H_3^2 - N^2 - N^{15} dihydrochloride, m.p. $188-190^\circ$ (cor.).

Resolution of Isotopic DL-Lysine Dihydrochloride. DL-Lysine is resolved with D- and L-camphoric acid according to Berg,¹ as follows.

To 76.7 g. (0.35 mole) of DL-lysine dihydrochloride, dissolved in approximately 200 ml. of water, is added slightly over 0.35 mole of freshly prepared silver oxide. The mixture is stirred to facilitate precipitation of the silver chloride, the latter is collected on a filter, and excess silver is removed from the filtrate as the sulfide. The clear filtrate is partially concentrated *in vacuo*, 35.0 g. (0.175 mole) of D-camphoric acid dissolved in methyl alcohol is added, and concentration of the solution is carried to dryness. The residue displays $[\alpha]_D^{20} +7.94^\circ$ (Note 1).

The mixture of L(+)- and D(-)-lysine-3,4,5- $H_3^2-N^2-N^{15}$ D-camphorates is recrystallized from a minimal volume of a mixed solvent: 3 parts methyl alcohol and 2 parts water (Note 2). The mother liquor is concentrated to incipient crystallization; then an equal volume of methyl alcohol is added. This process is repeated as long as fractions of appreciable size are obtained (Note 3). Each of the more insoluble fractions is similarly refractionated (Note 4). Fraction I, 8.6 g., shows $[\alpha]_D^{20} +15.33^\circ$; Fraction II, 28.5 g., $[\alpha]_D^{20} +13.93^\circ$; Fraction III, 48.7 g., $[\alpha]_D^{20} +3.2^\circ$. On recrystallization, Fraction I yields 8.1 g., $[\alpha]_D^{20} +16.14^\circ$, and Fraction II, 20.4 g., $[\alpha]_D^{20} +16.35^\circ$. These fractions are combined and recrystallized to yield 22.53 g. of optically pure L(+)-lysine D-camphorate, $[\alpha]_D^{20} +16.41^\circ$, m.p. 245–246° (cor.); 52.7% based on 76.7 g. of DL-lysine dihydrochloride (Note 5).

The fractions showing rotations of $+4.5^\circ$ or less are combined with the dry residues from the various mother liquors, dissolved in water and made acidic (Congo red) with sulfuric acid. The D-camphoric acid is extracted with ether, and barium hydroxide is added carefully to effect the exact removal of sulfate ions. The calculated amount of L-camphoric acid, needed to replace the D-camphoric acid removed, is added, and the solution is evaporated to dryness. Fractionation of the L-camphorate mixture, essentially as outlined for the D-camphorate, yields 27.79 g. (64.5%) of D(-)-lysine-3,4,5- $H_3^2-N^2-N^{15}$ L-camphorate, with rotation $[\alpha]_D^{20} -16.39^\circ$, m.p. 245–246° (cor.) (Note 6).

(c) L(+)-Lysine-3,4,5- $H_3^2-N^2-N^{15}$ Dihydrochloride. To 19.7 g. (0.04 mole) of L(+)-lysine D-camphorate dissolved in 100 ml. of water is added 20 ml. of concentrated hydrochloric acid. The bulk of the camphoric acid liberated is collected on a filter, and the remainder is extracted with ether. The lysine hydrochloride solution is concentrated *in vacuo* to a thin syrup, which is warmed on the steam-bath and dissolved in a minimum amount of alcohol. Lysine dihydrochloride is precipitated by addition of 3 to 5 volumes of acetone to the solution. The precipitate is redissolved and again precipitated to obtain 17.1 g. (97.6%) of L(+)-lysine-3,4,5- $H_3^2-N^2-N^{15}$ dihydrochloride, m.p. 201–202° (cor.), $[\alpha]_D^{20} +15.63^\circ$.

(d) D(-)-Lysine-3,4,5- $H_3^2-N^2-N^{15}$ Dihydrochloride. Treatment of 19.7 g. of D(-)-lysine L-camphorate in the same manner as the L(+)-lysine D-camphorate, above, yields 17.16 g. (97.9%) of D(-)-lysine-3,4,5- $H_3^2-N^2-N^{15}$ dihydrochloride, m.p. 201–202°, $[\alpha]_D^{20} -15.65^\circ$.

B. Notes

1. Aqueous solutions of the camphorates, 2.00 g. per 100 ml., are employed throughout.

2. This ratio of solvents was recommended to Weissman and Schoenheimer² by Berg.

3. Usually, crystallizations are allowed to continue overnight at low temperature.

4. Such a procedure must necessarily be followed closely by optical measurements, since practically unavoidable variations in technique influence the course of the fractionation.

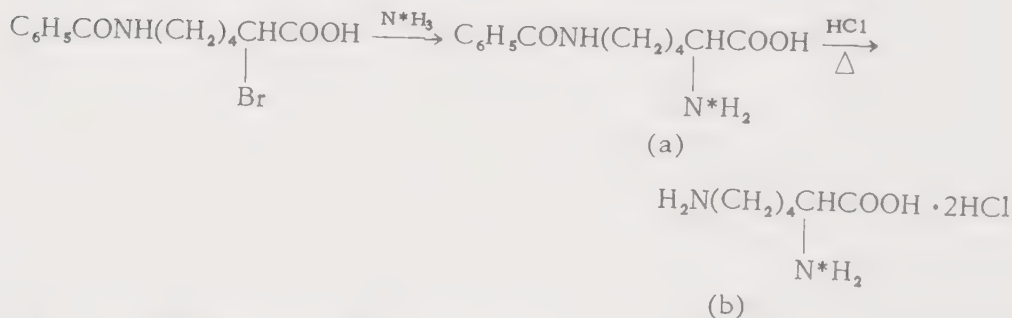
5. Further recrystallization did not change the rotation.

6. A procedure for further processing of the mother liquors is given.¹ The final yields of the two isomeric camphorates are about 80%.

¹C. P. Berg, J. Biol. Chem., 115, 9 (1936).

²N. Weissman and R. Schoenheimer, *ibid.*, 140, 786 (1941).

L-LYSINE-*N*²-*N*¹⁵ DIHYDROCHLORIDE
(L-2,6-Diaminohexanoic-2-*N*¹⁵ Acid Dihydrochloride)



H. R. V. Arnstein, G. D. Hunter, H. M. Muir and A. Neuberger, J. Chem. Soc., 1952, 1329.

A. Procedure

(a) *N*⁶-Benzoyl-L-lysine-*N*²-*N*¹⁵, (L-2-Amino-6-benzamidohexanoic-2-*N*¹⁵ Acid). To a solution containing 0.95 g. of ammonia-*N*¹⁵ is added 7.3 g. of D-6-benzamido-2-bromohexanoic acid (Note 1). The flask is tightly stoppered and kept at 40° for 4 days. The solution is acidified to pH 6 with hydrochloric acid, and the precipitated solid is collected and dried; yield, 4.24 g. (73%). The crude product is recrystallized by dissolution in hydrochloric acid and addition of sodium acetate. (Note 2).

(b) L-Lysine-*N*²-*N*¹⁵ Dihydrochloride, (L-2,6-Diaminohexanoic-2-*N*¹⁵ Acid Dihydrochloride). The benzamido compound, 3.0 g., is hydrolyzed by heating under reflux with concentrated hydrochloric acid, which is then removed by evaporation; the residue is dissolved in water and again evaporated to dryness. The residue is dissolved in water and the solu-

tion is adjusted to pH 6. To this solution is added 1 equivalent of sodium hydroxide and 3.0 g. of picric acid. The mixture is heated until a clear solution is obtained. The crystalline picrate, obtained upon cooling the solution to 0°, is recrystallized from water. To a solution of L-lysine- N^2 - N^{15} picrate, 9.0 in 100 ml. of hot water, is added 5 ml. of hydrochloric acid, and the solution is continuously extracted with hot benzene until the aqueous layer is no longer yellow. The L-lysine- N^2 - N^{15} salt, obtained by evaporation of the aqueous layer to dryness, is crystallized from aqueous ethanol by the addition of pyridine. The product is recrystallized from aqueous ethanol and appears first as the monohydrochloride monohydrate, and then as the dihydrochloride; $[\alpha]_D^{20}$ (in terms of lysine) + 22.4° (c, 4.00 in 5% HCl).

B. Notes

1. D-6-Benzamido-2-bromohexanoic acid is prepared from D-lysine.¹ The reaction of an α -bromo acid with ammonia is generally a nucleophilic bimolecular substitution and, thus, inversion of configuration occurs.^{2,3} In the conversion of N^6 -benzoyl-D-lysine into 6-benzamido-2-bromohexanoic acid, the D-configuration is retained; therefore, the inversion affords the L-lysine isomer.

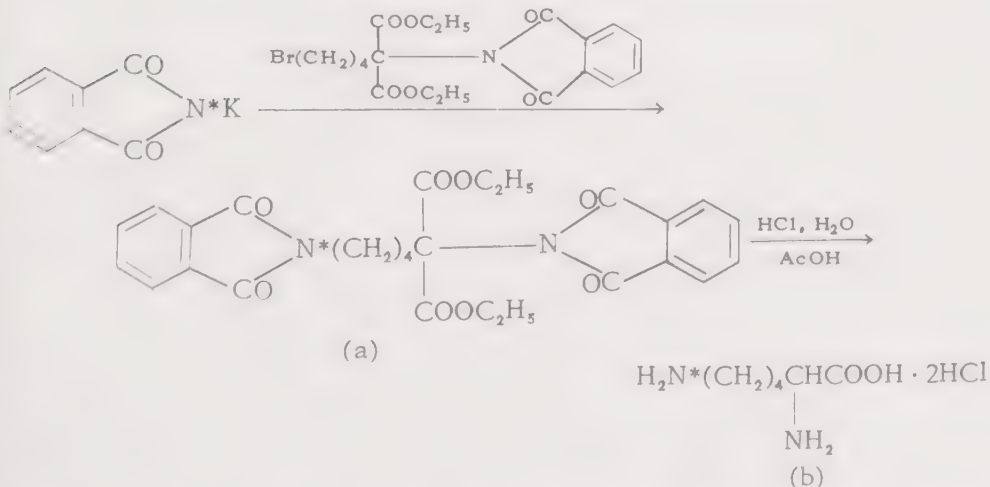
2. The excess of ammonia- N^{15} is recovered by addition of alkali to the first mother liquor and distillation of the ammonia into dilute sulfuric acid.

¹A. Neuberger and F. Sanger, *Biochem. J.*, 38, 232 (1944).

²A. Neuberger, *Adv. Protein Chem.*, 4, 297 (1948).

³P. Brewster, E. D. Hughes, C. K. Ingold and P. A. D. S. Rao, *Nature*, 166, 178 (1950).

LYSINE- N^6 - N^{15} DIHYDROCHLORIDE (2,6-Diaminohexanoic-6- N^{15} Acid Dihydrochloride)



R. M. Fink, T. Enns, C. P. Kimball, H. E. Silberstein, W. F. Bale, S. C. Madden and G. H. Whipple, *J. Exptl. Med.*, 80, 457 (1944).

A. Procedure

(a) 2-Carbethoxy-N²,N⁶-Diphtaloyllysine-N⁶-N¹⁵ Ethyl Ester. Ethyl α -(4-bromobutyl)-1,3-dioxo-2-isoinolinemalonate (Note 1), 79 g. (0.18 mole), is stirred and heated for 5 hours at 150–160° with 29.4 g. (0.16 mole) of potassium phthalimide-N¹⁵. The product is extracted with three 200-ml. portions of hot absolute alcohol, which is filtered while hot and concentrated to a thick syrup *in vacuo*.

(b) Lysine-N⁶-N¹⁵ Dihydrochloride, (2,6-Diaminohexanoic-6-N¹⁵ Acid Dihydrochloride). The syrupy diphthalimido compound is heated under reflux for 5 hours with a mixture of 400 ml. each of concentrated hydrochloric acid, glacial acetic acid and water. About 300 ml. of the mixture is removed by distillation, and refluxing is then continued for 24 hours. The mixture is cooled, filtered and concentrated *in vacuo* to about 100 ml. The residue is dissolved in 120 ml. of hot absolute alcohol, and 1200 ml. of acetone is added slowly. The lysine-N⁶-N¹⁵ dihydrochloride crystallizes during 2 days in the refrigerator. The yield is 26.5 g. (67%). The crude product is dissolved in 500 ml. of hot absolute alcohol and filtered; crystallization is effected by the addition of 700 ml. of ether. The yield is 17.5 g. (44.2%) (Note 2).

B. Notes

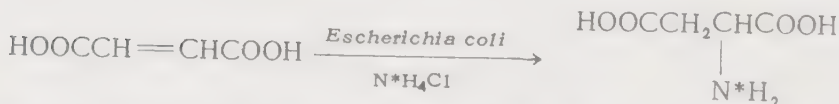
1. The preparation of this compound from ethyl sodiumphthalimidomalonate¹ and tetramethylene bromide is given by Fink, et al.

2. Conversion to the monohydrochloride² occasionally gives better yields and a purer product than does a second recrystallization as the dihydrochloride.

¹M. S. Dunn and B. W. Smart, J. Biol. Chem., 89, 41 (1930).

²Organic Syntheses, Vol. 19, Wiley, New York, 1939, p. 62.

L-ASPARTIC-N¹⁵ ACID
(L-2-Aminosuccinic-N¹⁵ Acid)



H. Wu and D. Rittenberg, J. Biol. Chem., 179, 847 (1949).

A. Procedure (Note 1)

A suspension of 15 g. of fumaric acid in water is neutralized with sodium hydroxide, and the resulting solution is diluted to 130 ml. To this solution are added: 4 g. of ammonium-N¹⁵ chloride, 100 ml. of Clark and Lubs 0.05 M phosphate buffer (pH 7.4), 25 ml. of *E. coli* suspension

(Note 2), and 20 ml. of toluene. The flask containing the mixture is evacuated, filled with nitrogen and placed in an incubator at 37° (Note 3). After 2 days, the mixture is boiled and centrifuged, and the precipitate is washed with hot water. To the combined solutions is added 60 ml. of saturated copper sulfate solution, and the mixture is refrigerated overnight. The crystals of copper L-aspartate-N¹⁵ are collected, washed with a little cold water, suspended in 600 ml. of hot water and treated with hydrogen sulfide. Copper sulfide is removed by filtration; the filtrate is concentrated to about 150 ml. and diluted with 2 volumes of 95% alcohol. After several hours, the crystalline L-aspartic-N¹⁵ acid is collected and washed with alcohol and ether; yield, 6 g.

B. Notes

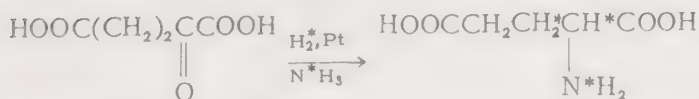
1. Quastel and Woolf¹ have shown that *Escherichia coli* catalyzes the reversible reaction between fumarate and ammonia producing L-aspartic acid. A strictly chemical synthesis of L-aspartic-N¹⁵ acid has not been described.

2. *E. coli* was grown on 2% agar in 10 Roux bottles. The agar medium contained 2% of Bacto-tryptone and 0.5% each of glucose, sodium chloride and concentrated yeast extract. After 2 days, the culture of *E. coli*, which was separated from the agar by gentle agitation with normal saline solution, was filtered through gauze, centrifuged, washed and made up to 200 ml. with normal saline solution.

3. The ammonia content of the mixture fell to 19.2% of the original in 24 hours, and to 18% in 48 hours; apparently equilibrium was reached in about 2 days, under the conditions employed.

¹J. H. Quastel and B. Woolf, *Biochem. J.*, 20, 545 (1926).

GLUTAMIC-2,3-H₃²-N¹⁵ ACID (2-Aminoglutaric-2,3-H₃²-N¹⁵ Acid)



S. Ratner, *J. Biol. Chem.*, 152, 559 (1944).

A. Procedure

2-Oxoglutaric acid¹ is hydrogenated with deuterium gas in the presence of ammonia-N¹⁵ by modification (Note 1) of the general procedure of Schoenheimer and Ratner² for preparing N¹⁵-labeled α-amino acids; see glutamic-N¹⁵ acid. The deuterium in the isolated product is located in the 2 and 3 positions (Note 2).

B. Notes

1. Deuterium gas was substituted for the ordinary hydrogen in the usual procedure.

2. It has been shown by Ratner³ and Rittenberg⁴ that glutamic-2,3-H₂² acid, prepared by catalytic hydrogenation of 2-oxoglutaric acid, contains stably bound deuterium in both the 2- and 3-positions. None of the deuterium was removed on prolonged boiling of the sample with 20% hydrochloric acid; therefore, there was no deuterium in the 4-position, since deuterium in the 4-position was shown to exchange under these conditions.

C. Other Preparations

Aspartic-2,3-H₂²-N¹⁵ acid has been prepared⁵ from oxalacetic acid according to the modification described in the procedure of Schoenheimer and Ratner² for the preparation of N¹⁵-labeled amino acids; see aspartic-N¹⁵ acid.

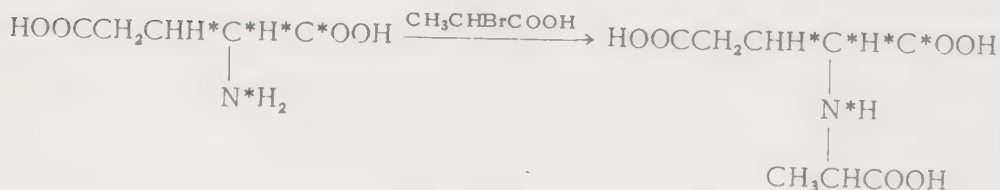
¹C. Neuberg and M. Ringer, *Biochem. Z.*, **71**, 228 (1915).

²R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, **127**, 301 (1939).

³S. Ratner, D. Rittenberg and R. Schoenheimer, *ibid.*, **135**, 357 (1940).

⁴D. Rittenberg, S. Ratner and H. D. Hoberman, *J. Am. Chem. Soc.*, **62**, 2249 (1940).

⁵F. Kögl, P. Emmelot and D. H. W. den Boer, *Ann.*, **589**, 1 (1954).

C₂¹⁴-H₂²-N¹⁵-N-(1-CARBOXYETHYL)GLUTAMIC ACID

F. Kögl and J. de Flines, *Rec. trav. chim.*, **72**, 1009 (1953).

A. Procedure

A solution containing 0.7 g. of C₂¹⁴-H₂²-N¹⁵-L-glutamic acid hydrochloride (3.8 mmoles) (Note 1), 1.07 g. of D-2-bromopropionic acid (7.0 mmoles) and 1.03 g. of sodium hydroxide in 14 ml. of water is warmed at 37° for 8 days. The reaction mixture is then fractionated by passage through a column of Amberlite ion exchange resin (29 g.; 55 cm. × 1.2 cm.). Solvent water is passed through the column at the rate of 40 ml. per hour. The first fraction of about 200 ml. contains hydrobromic, hydrochloric and lactic acids. After about 280 ml., the effluent is radioactive (Note 2), and the following 720 ml. is collected, concentrated

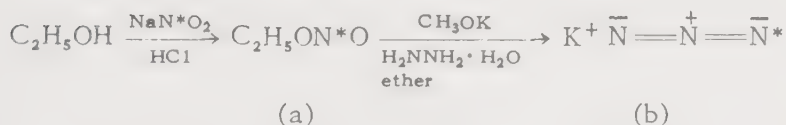
under vacuum to 5 ml. and diluted with 75 ml. of acetone. After the solution is kept at 0° overnight, the crystalline product is collected and twice recrystallized. The yield of product, m.p. 140–142° (dec.), is 572 mg. (67%); $[\alpha]_D^{20} + 22.0^\circ$ (c, 5.00 in 10% hydrochloric acid).

B. Notes

1. The $C_2^{14}-H_2-N^{15}$ -L-glutamic acid hydrochloride was actually a mechanical mixture of L-glutamic-1,2- $C_2^{14}-N^{15}$ acid hydrochloride with L-glutamic-2,3- H_2 acid hydrochloride which was recrystallized from 20% hydrochloric acid.

2. A paper chromatogram showed the presence of the desired product.

POTASSIUM AZIDE-1- N^{15}



K. Clusius and H. Hurzeler, *Helv. Chim. Acta*, 36, 1326 (1953).

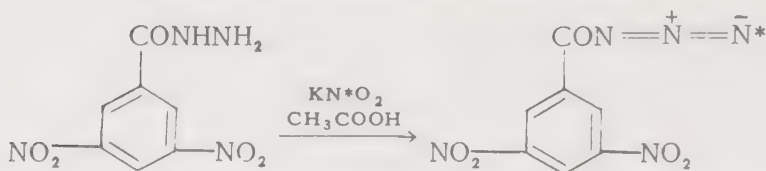
A. Procedure

(a) *Ethyl Nitrite- N^{15}* . A solution of 2.6 g. of sodium nitrite- N^{15} in 5.3 ml. of water is mixed with 4.5 ml. of ethanol and added dropwise to 3.2 ml. of concentrated hydrochloric acid which is cooled in an ice-bath. The ethyl nitrite- N^{15} is distilled from a water-bath at 30° through a small tube filled with anhydrous potassium carbonate into a flask cooled in ice; yield, 2.8 ml.

(b) *Potassium Azide-1- N^{15}* (Note 1). To a solution of 2.2 g. of potassium in 14 ml. of methyl alcohol, cooled in ice, are added 28 ml. of dry ether, 2.8 ml. of hydrazine hydrate and 2.8 ml. of ethyl nitrite- N^{15} . The mixture is kept for a time in an ice-bath and then at room temperature for 24 hours. The yield of crystalline potassium azide-1- N^{15} , which is washed with methanol-ether and ether, is 2.2 g. (72% based on sodium nitrite- N^{15}).

B. Notes

1. Isotopic analyses of the reduction and oxidation products formed from the labeled azide ion proved conclusively: that only one nitrogen atom in each ion was labeled; that the configuration is linear and not a ring; and that the labeled atom was terminal. Since, in the azide ion, $\bar{N} - \overset{+}{N} - \bar{N}^*$ the two end atoms are indistinguishable, the name is azide-1- N^{15} .

3,5-DINITROBENZOYL AZIDE-3-N¹⁵

A. A. Bothner-By and L. Friedman, *J. Am. Chem. Soc.*, 73, 5391 (1951).

A. Procedure

3,5-Dinitrobenzoyl azide-3-N¹⁵ is prepared according to the following procedure of Sah and Ma.¹

3,5-Dinitrobenzhydrazide, 10 g., is dissolved in 100 ml. of glacial acetic acid. With continuous stirring and efficient cooling, a solution of 8 g. of sodium nitrite in 25 ml. of water is added dropwise (Note 1). After all the nitrite solution is added, the reaction mixture is stirred for a few minutes more, and the 3,5-dinitrobenzoyl azide is precipitated in fine white needles by the addition of 300 ml. of water. The solid is collected, washed well with water and dried over sulfuric acid in a vacuum desiccator. The yield is nearly quantitative (11 g.). 3,5-Dinitrobenzoyl azide is quite stable and, when thoroughly dried, melts at 87–89° with a slow evolution of gas (Note 2). It is insoluble in water, slightly soluble in cold alcohol, petroleum ether and carbon tetrachloride, but very soluble in these solvents when hot. It is also very soluble in acetone, ethyl acetate, chloroform, benzene, toluene and glacial acetic acid.

The yield of 3,5-dinitrobenzoyl azide-3-N¹⁵ (Note 2) is 0.412 g. (89.7%) using 0.171 g. of potassium nitrite-N¹⁵ in the diazotization.

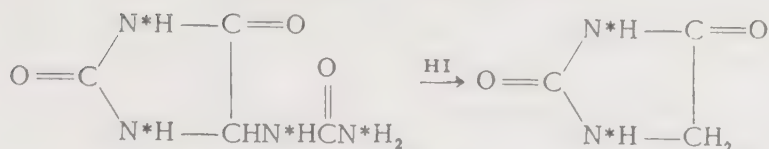
B. Notes

1. A white precipitate forms immediately, but partially redissolves until the solution becomes saturated with the azide.

2. Thermal decomposition of the azide indicated that all the nitrogen-15 was present as the terminal nitrogen. Mass spectrometric analysis of the evolved nitrogen showed it to be 31.1 atom % N¹⁴N¹⁵ and 0.1 atom % N₂¹⁵. The 3,5-dinitroaniline, resulting from hydrolysis of the isocyanate formed in the decomposition of the azide, contained no excess nitrogen-15.

¹P. P. T. Sah and T. S. Ma, *J. Chinese Chem. Soc.*, 2, 159 (1938).

HYDANTOIN-N¹⁵₂
(2,4-Imidazolidinedione-N¹⁵₂)



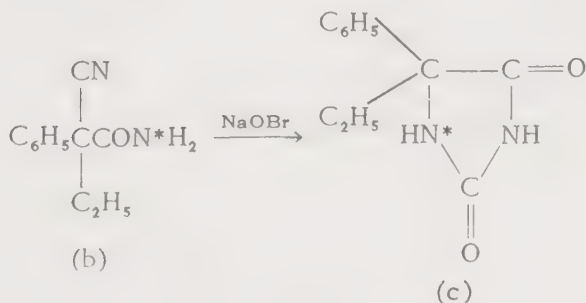
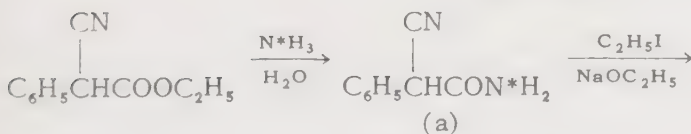
L. F. Cavalieri and G. B. Brown, J. Am. Chem. Soc., 70, 1242 (1948).

Procedure

The procedure of Baeyer¹ for the reduction of allantoin to hydantoin is simplified somewhat. 5-(Ureido-1,3-N¹⁵_{2/1})hydantoin-1,3-N¹⁵_{2/1}, (N¹⁵₂-Allantoin), 0.2 g., is heated under reflux for 8 minutes with 2 ml. of hydriodic acid, containing a trace of hypophosphite. The mixture is then evaporated to dryness with a stream of air. Three ml. of acetone is added to the dry residue, and the solution is refrigerated for 0.5 hour. The hydantoin, 0.7 g., is collected on a filter, washed with acetone and recrystallized from ethanol. The yield of hydantoin-N¹⁵₂ is 0.57 g. (45%).

¹A. Baeyer, Ann., 130, 158 (1864).

5-ETHYL-5-PHENYLHYDANTOIN-1-N¹⁵
(N¹⁵₁-Nirvanol)



H. Sobotka and F. E. Stynler, J. Mt. Sinai Hosp., 19, [1], 212 (1952).

A. Procedure

(a) 2-Cyano-2-phenylacetamide-N-N¹⁵. Ethyl cyanophenylacetate^{1,2} is converted to the amide according to the procedure of Hessler³ (Note 1).

The ammonia- N^{15} from ammonium- N^{15} nitrate is distilled into an aqueous suspension of the ethyl ester. After 24 hours, the crystalline amide- N^{15} is collected, washed with water and dried; yield, 62%. The amide, practically insoluble in water, is recrystallized from absolute alcohol, m.p. 147° .

(b) 2-Cyano-2-phenylbutyramide- $N-N^{15}$. The 2-cyano-2-phenylacetamide- $N-N^{15}$ in absolute alcohol is treated with an alcoholic solution of 1 equivalent of sodium ethoxide; ethyl iodide is then added in 25% molar excess.^{4,5} The temperature rises and, after a time, the solution becomes neutral to litmus. After most of the alcohol and the excess ethyl iodide are removed by distillation, the residue is washed with water and recrystallized from alcohol. The yield of 2-cyano-2-phenylbutyramide- $N-N^{15}$ is 87% (Note 2).

(c) 5-Ethyl-5-phenylhydantoin-1- N^{15} , (N_1^{15} -Nirvanol). The 2-cyano-2-phenylbutyramide- $N-N^{15}$ is subjected to oxidative ring closure with sodium hypobromite to obtain 5-ethyl-5-phenylhydantoin-1- N^{15} in 91% yield, m.p. 201° (Note 3). According to the patent literature,⁴ the amide is dissolved in sodium hypobromite solution, and after the solution is warmed a short time the hydantoin is formed. After recrystallization from alcohol, the product melts at $201-202^{\circ}$.

B. Notes

1. Hessler³ prepared 2-cyano-2-phenylacetamide, in 67.2% yield by treating ethyl cyanophenylacetate with concentrated ammonium hydroxide at room temperature.

2. The melting point, after recrystallization from alcohol, is 116° .⁴

3. According to Sobotka and Stynler, the Hofmann reaction must be carried out using the special conditions given in *Organic Reactions*.⁶

¹M. F. Bodroux, Bull. soc. chim., (IV) 9, 651 (1911).

²W. L. Nelson and L. H. Cretcher, J. Am. Chem. Soc., 50, 2758 (1928).

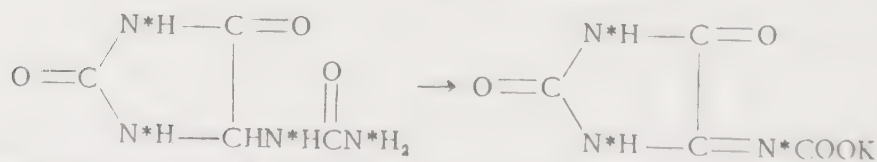
³J. C. Hessler, Am. Chem. J., 32, 122 (1904).

⁴Chem. Fabrik von Heyden A. G., D.R.P., 309,508, May 5, 1914; through Chem. Zentr., 1919, II, 262.

⁵J. C. Hessler, Am. Chem. J., 22, 169 (1899).

⁶*Organic Reactions*, Vol. III, Wiley, New York, 1948, p. 282.

5-CARBOXYIMINO- $N_{1/1}^{15}$ -HYDANTOIN-1,3- $N_{2/1}^{15}$ POTASSIUM SALT (Potassium N^{15} -Allantoxanate)



L. F. Cavalieri and G. B. Brown, J. Am. Chem. Soc., 70, 1242 (1948).

A. Procedure

5-Ureido-1,3- $N_{2/1}^{15}$ -hydantoin-1,3- $N_{2/1}^{15}$ (N_2^{15} -Allantoin) is oxidized to 5-carboxyimino- $N_{1/1}^{15}$ -hydantoin-1,3- $N_{2/1}^{15}$ potassium salt according to the method of Biltz and Giesler,¹ as follows. To an ice-cold solution of 100 g. of allantoin in 500 ml. of 12% potassium hydroxide is added a cold solution of 64 g. of potassium permanganate in 1 l. of water made strongly alkaline by the addition of 350 ml. of 12% potassium hydroxide. After 3 hours, the manganese dioxide is removed by filtration, and the clear filtrate is acidified with acetic acid. The potassium oxonate forms a matted mass of crystals; yield, 77 g. (62%). The salt is readily recrystallized from a 17-fold amount of hot water (Note 1). Upon heating the salt in a melting point tube, it turns yellow at about 260°, brown at about 290° and decomposes a little above 360°.

In the isotopic synthesis, 0.085 g. of potassium N^{15} -allantoxonate is obtained from 0.1 g. of N_2^{15} -allantoin. Recrystallization from water gives 0.05 g. (40%) of purified product.

B. Notes

1. Solubility of the potassium salt in water at the boiling point is about 7 times that at room temperature. In alcohol and the other usual solvents, the salt is not particularly soluble.

C. Other Preparations

Brandenberger² has oxidized uric-7- N^{15} acid (biologically synthesized) with hydrogen peroxide in alkaline solution^{3,4} and isolated potassium N^{15} -allantoxanate in about 41% yield, after recrystallization from water. The oxidation of several C^{14} -labeled uric acids was included in this work, which indicated that the oxidation product did not contain the original imidazole ring of the uric acid molecule. Brandenberger² suggests a symmetrical triazine, 4,6-dihydroxy-*s*-triazine-2-carboxylic acid, as the structure of allantoxanic acid; this is supported by the fact that allantoxanic acid can be converted to cyanuric acid.⁵

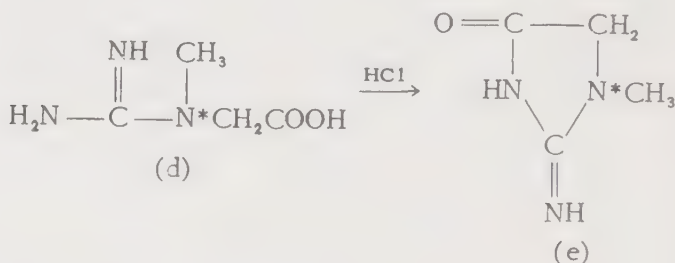
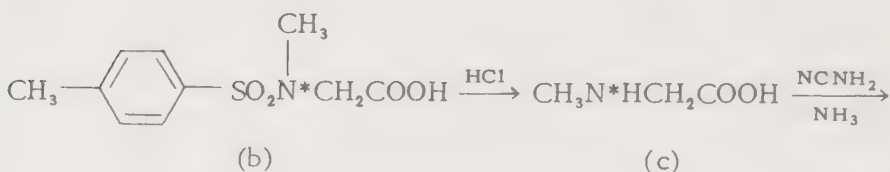
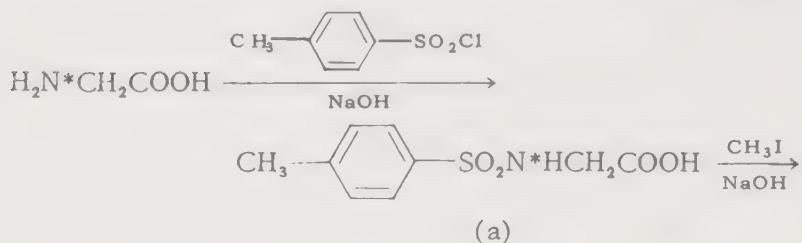
¹H. Biltz and E. Giesler, *Ber.*, 46, 3413 (1913).

²H. Brandenberger, *Biochem. and Biophys. Acta*, 15, 108 (1954).

³C. S. Venable, *J. Am. Chem. Soc.*, 40, 1099 (1918).

⁴F. J. Moore and R. Thomas, *ibid.*, 40, 1120 (1918).

⁵H. Brandenberger, *Helv. Chim. Acta*, 37, 641 (1954).

CREATININE-1-N¹⁵(2-Imino-1-methyl-4-imidazolidinone-1-N¹⁵)

K. Bloch and R. Schoenheimer, J. Biol. Chem., 131, 116 (1939).

A. Procedure

(a) *N-p-Toluenesulfonylglycine-N¹⁵*. Glycine-N¹⁵ is converted to *N-p*-toluenesulfonylglycine-N¹⁵ according to the following procedure of Fischer and Bergmann.¹ A mixture of glycine, *p*-toluenesulfonyl chloride and a corresponding amount of 2 *N* sodium hydroxide is shaken at 67–70°. Upon acidification of the alkaline solution, the product crystallizes in fine needles, melting at 149–150° (cor.) to a clear liquid (Note 1).

(b) *N-p-Toluenesulfonylsarcosine-N¹⁵*, (*N-Methyl-N-p-toluenesulfonylglycine-N¹⁵*). According to Fischer,¹ 38 g. of *N-p*-toluenesulfonylglycine is dissolved in 200 ml. of 3 *N* sodium hydroxide solution and shaken with 28 g. of methyl iodide in a closed flask immersed in a bath at 67°. After 10 minutes, a clear solution results which is kept at 67° for an additional 50 minutes. Upon acidification of the cooled solution, an oil separates which soon crystallizes with ice-bath cooling. The product is dissolved in potassium bicarbonate, precipitated by acidification of the solution and finally recrystallized from 1 liter of hot water. The yield of dry product is 38 g. (Note 2).

(c) *Sarcosine-N¹⁵*, (*N-Methylglycine-N¹⁵*). According to Fischer,¹ 10 g. of *N*-methyl-*N*-*p*-toluenesulfonylglycine is heated with 40 ml. of concentrated hydrochloric acid in a sealed tube at 100° for 22 hours. The acidic solution is cooled to 0°, the slightly soluble *p*-toluenesulfonic acid is filtered off, and the filtrate is evaporated to dryness. The crystalline residue is triturated with 20 ml. of alcohol, ether is added, and the crystalline product is collected and dried. The yield of sarcosine hydrochloride, m.p. 171–174° (cor.), is 4.7 g. (91%).

(d) *N₁¹⁵-Creatine*, (*1-Methylguanidino-1-N¹⁵-acetic Acid*). Sarcosine-*N¹⁵* is condensed with cyanamide according to an adaptation of the procedure of Rosengarten.² To a concentrated aqueous solution of the two reactants is added a trace of ammonium hydroxide. Upon standing, the solution deposits crystals of *N₁¹⁵-creatine*. The over-all yield of *N₁¹⁵-creatine*, based upon glycine-*N¹⁵*, is 58%, after recrystallization from water (Note 3).

(e) *Creatinine-1-N¹⁵*, (*2-Imino-1-methyl-4-imidazolidinone-1-N¹⁵*). *N₁¹⁵-Creatine* is heated under reflux with concentrated hydrochloric acid.³ The solution is then concentrated to dryness on a steam-bath, and creatinine-1-*N¹⁵* is recovered as the hydrochloride.

B. Notes

1. *N*-*p*-Toluenesulfonylglycine is quite soluble in alcohol and acetone and soluble in hot and slightly soluble in cold water.

2. The product may be recrystallized from acetone solution by the addition of petroleum ether, m.p. 150–152° (cor.). It is quite soluble in acetone, soluble in alcohol and hot benzene, and slightly soluble in petroleum ether.

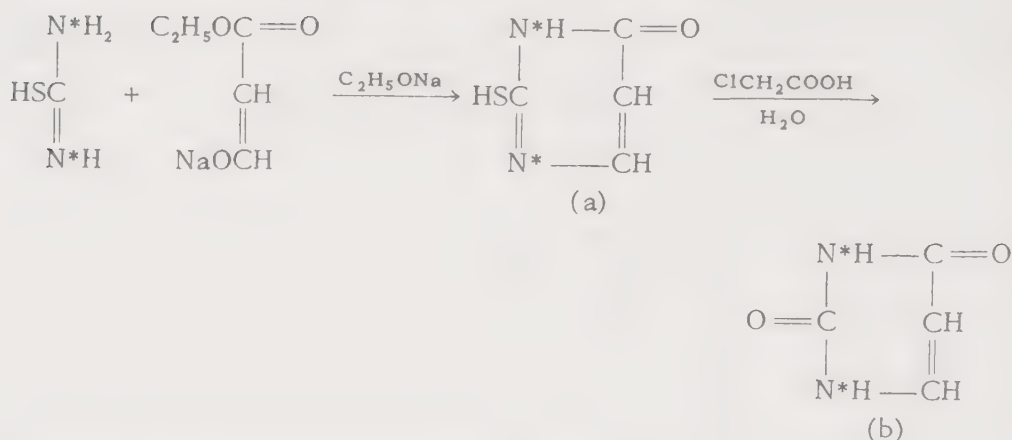
3. Creatine crystallizes from water as the monohydrate, which becomes anhydrous upon drying at 100°. Creatine picrate, pale-yellow needles from water, melts at 220–221°.

¹E. Fischer and M. Bergmann, *Ann.*, 398, 96 (1913).

²F. Rosengarten and A. Strecker, *Ann.*, 157, 1 (1871).

³*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 172.

URACIL- N_2^{15}
[2,4(1H,3H)-Pyrimidinedione- N_2^{15}]



A. A. Plentl and R. Schoenheimer, *J. Biol. Chem.*, **153**, 211 (1944).

A. Procedure

(a) *2-Thiouracil- N_2^{15}* . Thiourea- N_2^{15} , 2.14 g., is added to 100 ml. of 95% ethanol in which 1.5 g. of sodium has been dissolved. To this mixture, 12 g. of powdered ethyl sodiummalonaldehyde is added, and the solution is heated under reflux for 3.5 hours. At the end of this time, 20 ml. of water is added, and heating is continued 0.5 hour. The mixture is then evaporated to dryness under reduced pressure, and the residue is dissolved in 40 ml. of water, chilled in ice and acidified with 50% acetic acid. After remaining in the refrigerator overnight, the product is collected on a filter and recrystallized from 125 ml. of boiling water (Note 1). The yield of *2-thiouracil- N_2^{15}* is 1.57 g.

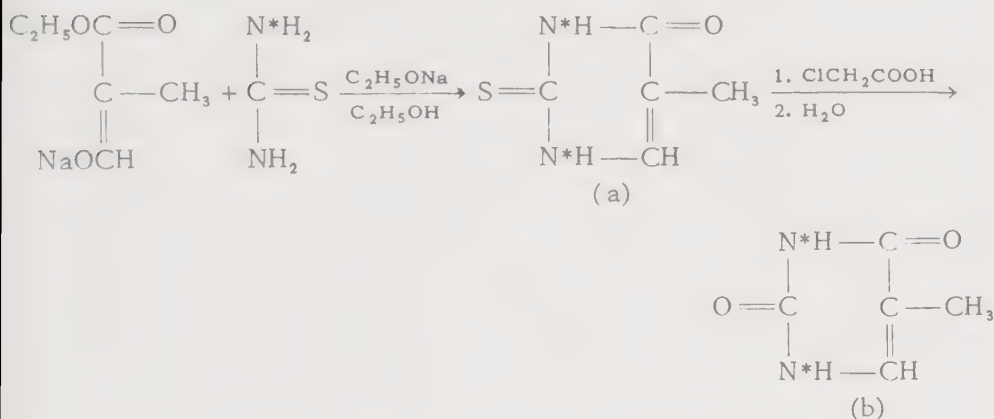
(b) *Uracil- N_2^{15}* . Uracil is prepared according to the procedure of Wheeler,¹ as follows. A mixture of 30 g. of 2-thiouracil, 33 g. of chloroacetic acid and 700 ml. of water is heated under reflux. When all the material has dissolved, the solution is evaporated to dryness on a steam-bath. The residue is warmed with alcohol, collected on a filter and washed with alcohol. It is then free of sulfur and weighs 23.5 g. (89.5%).

B. Notes

1. Carbon is used to remove colored by-products.

¹H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

THYMINE-1,3- $N_{1/2}^{15}$
[5-Methyl-2,4(1*H*,3*H*)-pyrimidinedione-1,3- $N_{1/2}^{15}$]



A. A. Plentl and R. Schoenheimer, J. Biol. Chem., 153, 203 (1944).

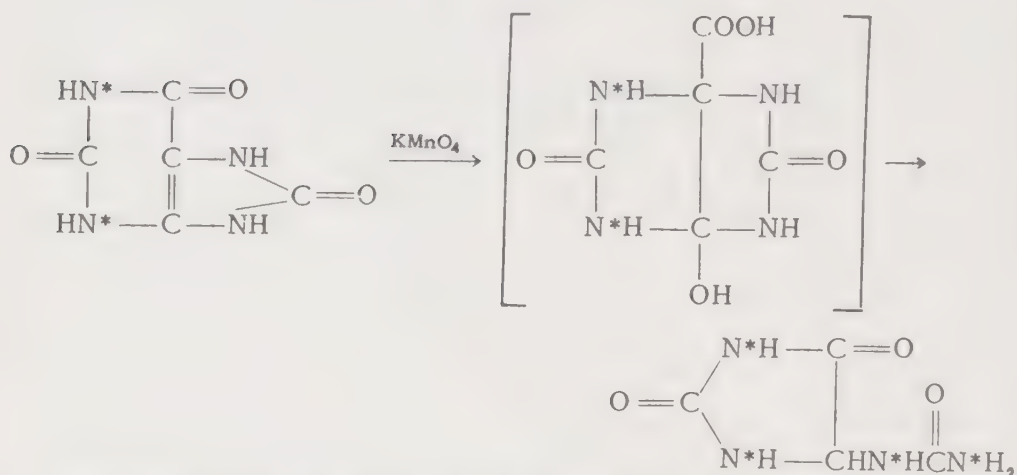
A. Procedure

(a) *Thiothymine-1,3- $N_{1/2}^{15}$* . Thiourea- N_1^{15} , 2.0 g., is added to 100 ml. of 95% ethanol in which 1.5 g. of sodium has been dissolved. To this mixture, 12 g. of ethyl methylsodiummalonaldehyde is added, and the solution is heated under reflux for 3.5 hours. At this time, 20 ml. of water is added, and heating is continued for 0.5 hour. The mixture is evaporated to dryness under reduced pressure, and the residue is redissolved in 40 ml. of water, chilled in ice and acidified with 50% acetic acid. After remaining in the refrigerator overnight, the product is collected and recrystallized twice from boiling water (Note 1). The yield of thiothymine-1,3- $N_{1/2}^{15}$ is 0.94 g.

(b) *Thymine-1,3- $N_{1/2}^{15}$* . The 0.94 g. of thiothymine-1,3- $N_{1/2}^{15}$ is desulfurized with chloroacetic acid according to the general method of Wheeler and Liddle¹ (see uracil-1,3- N_2^{15}).

¹H. M. Wheeler and L. M. Liddle, Am. Chem. J., 40, 547 (1908).

5-(UREIDO-1,3-N¹⁵_{2/1})HYDANTOIN-1,3-N¹⁵_{2/1}
(N¹⁵₂-Allantoin)



L. F. Cavalieri and G. B. Brown, J. Am. Chem. Soc., 70, 1242 (1948).

A. Procedure

Uric-1,3-N¹⁵ acid, 1.2 g., is oxidized to N¹⁵₂-allantoin (Note 1) according to the procedure of Hartman¹ (Note 2). In this method, 100 g. (0.595 mole) of uric acid and 4.5 l. of hot water (70–80°) are placed in a 12-l. round-bottomed flask equipped with a mechanical stirrer. The stirrer is started, and a solution of 80 g. (2 moles) of sodium hydroxide (Note 3) in 120 ml. of water is added. Stirring is continued until all the uric acid is in solution, after which the solution is cooled by means of water directed against the flask. With the temperature at 25–30°, 50 g. (0.32 mole) of potassium permanganate is added all at once (Note 4) to the vigorously stirred solution. Stirring is continued for 15 to 20 minutes, and the mixture is filtered at once through a large (19 cm.) Büchner funnel. The first fraction of filtrate containing a small amount of manganese dioxide is returned to the funnel. As soon as the filtrate becomes clear, it is collected in a 12-l. round-bottomed flask which contains 130 ml. (2.2 moles) of glacial acetic acid. The filtrate is tested with litmus, to be sure that it is acid, and evaporated to a volume of 1.5–2 l. on a steam-bath, under reduced pressure (20–30 mm.). The solution is cooled overnight, and the allantoin crystals are collected on a Büchner funnel. The allantoin is dissolved in 800–900 ml. of boiling water, treated with 5 g. of carbon and filtered rapidly. The filtrate is cooled overnight, and the allantoin is collected. The yield of product, melting at 230–231° (Note 5), is 60–71 g. (64–75%).

In the isotopic preparation of Cavalieri and Brown, 0.866 g. of crude N¹⁵₂-allantoin is obtained. Upon recrystallization from water, the yield is 0.647 g. (57%).

B. Notes

1. Actually no single molecule is labeled with N^{15} in all four of the positions shown. However, the symmetrical intermediate gives rise to a mixture of two labeled entities, and on a statistical basis there should be 50% of each. Schuler and Reindel² isolated the silver salt of an intermediate to which they assigned the symmetrical structure first postulated by Behrend.³ Degradation⁴ of the 5-(ureido-1,3- $N^{15}_{2/1}$)hydantoin-1,3- $N^{15}_{2/1}$ indicated that the N^{15} is equally distributed between the hydantoin and urea moieties.

2. Since no experimental details are given for the isotopic synthesis, the information given is from the reference.¹

3. The use of more than 80 g. of sodium hydroxide does not increase the yield, but, if not neutralized immediately upon completion of the reaction, it causes decomposition of some allantoin.

4. The potassium permanganate must be added rapidly, one to five minutes.

5. The melting point depends upon rate of heating. The melting point 228–230° is observed in a capillary tube in a bath heated slowly from room temperature. If the capillary is placed in a bath at 228°, the specimen melts at 233–234°.

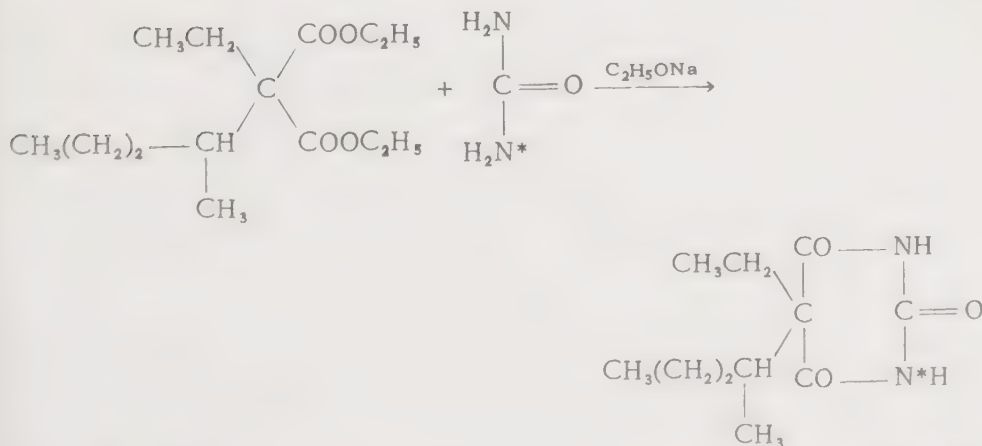
¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 21.

²W. Schuler and W. Reindel, *Z. physiol. Chem.*, 208, 248 (1932).

³R. Behrend, *Ann.*, 333, 146 (1904).

⁴L. F. Cavalieri and G. B. Brown, *J. Am. Chem. Soc.*, 70, 1242 (1948).

PENTOBARBITAL- N^{15}_1
[5-Ethyl-5-(1-methylbutyl)barbituric-1- N^{15} Acid]



H. B. van Dyke, J. V. Scudi and D. L. Tabern, *J. Pharmacol. Exptl. Therap.*, 90, 364 (1947).

A. Procedure

The condensation of ethyl ethyl(1-methylbutyl)malonate with urea- N_1^{15} to form pentobarbital¹ is done according to the Fischer and Dilthey² synthesis of alkybarbituric acids (Note 1). Metallic sodium (32 parts by weight) is dissolved in 600 parts of absolute alcohol; after cooling, 100 parts of dialkylmalonic ester is added, and 40 parts of powdered urea is dissolved with warming. The solution is heated 4-5 hours in an autoclave at 105-108°. The sodium salt of the acid and some sodium carbonate precipitate from the hot solution. After cooling to room temperature, the product is collected on a filter and washed with alcohol. Further heating of the alcoholic mother liquor results in a small amount of a purer product. The free acid is obtained by dissolving the sodium salt in water and decomposing it with concentrated hydrochloric acid.

The N^{15} preparation is purified by precipitation from a solution of the sodium salt with carbon dioxide.

B. Notes

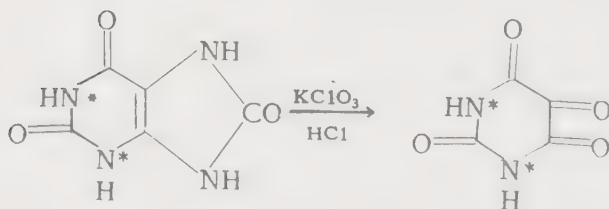
1. The synthesis of 5-ethyl-5-isopentylbarbituric-1- N^{15} acid by the same general procedure, is reported by Maynert and van Dyke.³

¹E. H. Volwiler and D. L. Tabern, J. Am. Chem. Soc., 52, 1676 (1930).

²E. Fischer and A. Dilthey, Ann., 335, 334 (1904).

³E. W. Maynert and H. B. van Dyke, J. Pharmacol. Exptl. Therap., 98, 180 (1950).

ALLOXAN- N_2^{15}
[2,4,5,6(1H,3H)-Pyrimidinetetrone- N_2^{15}]



L. F. Cavalieri and G. B. Brown, J. Am. Chem. Soc., 70, 1242 (1948).

A. Procedure

Using an adaptation of the procedure of Nightingale,¹ uric-1,3- N_2^{15} acid, 0.4 g., is treated with 0.107 g. of potassium chlorate, 0.67 ml. of concentrated hydrochloric acid and 1.1 ml. of water. After all the solid is in solution, the volume is reduced to about one-half by evaporation with a stream of air. The solution is cooled in the refrigerator overnight, and 0.1 g. of alloxan- N_2^{15} crystallizes.

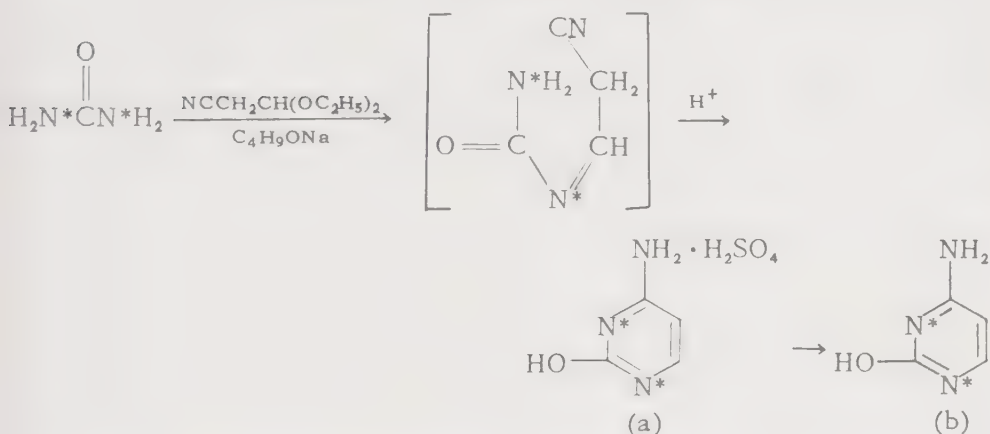
B. Other Preparations

Cavalieri and Brown have also prepared alloxan- N_2^{15} by oxidation of uric-1,3- N_2^{15} acid with nitric acid, which is similar to the procedure of Hartman² for the oxidation of alloxantin to alloxan.

¹*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 6.

²*Ibid.*, p. 3.

CYTOSINE-1,3- N_2^{15}
(4-Amino-2(1H)-pyrimidinone-1,3- N_2^{15})



A. Bendich, H. Getler and G. B. Brown, *J. Biol. Chem.*, 177, 565 (1949).

A. Procedure (Note 1)

(a) *Cytosine-1,3- N_2^{15} Sulfate*. In a three-necked flask, equipped with a sealed mechanical stirrer and a condenser protected from atmospheric moisture, 2.76 g. of sodium is dissolved in 90 ml. of anhydrous butanol. To the sodium butoxide solution are added 7.20 g. of dry urea and 17.4 ml. of freshly distilled cyanoacetaldehyde diethyl acetal (Note 2). The mixture is refluxed for 2 hours with mechanical stirring, which is necessary to prevent bumping as the sodium salt of the intermediate compound is deposited (Note 3). After chilling the mixture, the sodium salt is collected and washed with cold butanol and ether. The filtrate and washings are combined and concentrated to dryness *in vacuo*. The residue and main fraction of sodium salt are dissolved in 150 ml. of hot 2 *N* sulfuric acid. The hot solution is treated with carbon and filtered, and 2 volumes of hot ethanol are added to the filtrate. Upon cooling the solution 16.8 g. of cytosine sulfate crystallizes. The product may be recrystallized from a mixture of 2 *N* sulfuric acid and 95% ethanol (1:2, v/v) and dried at 139° over phosphorus pentoxide, *in vacuo* (Note 4).

(b) *Cytosine-1,3- N_2^{15}* . A solution of 11.8 g. of cytosine sulfate in a minimum of hot water is made alkaline with ammonium hydroxide and

decolorized with carbon. The filtrate is carefully adjusted to pH 7.0-7.5 with glacial acetic acid. Upon cooling the solution, 4.32 g. of cytosine is deposited in white plates (Note 5).

B. Notes

1. Cytosine-1,3- N_2^{15} was prepared according to the following procedure, described by Bendich, Getler and Brown, for nonisotopic cytosine. Liberated ammonia- N^{15} was collected in a boric acid trap during the condensation.

2. Cyanoacetaldehyde diethyl acetal was prepared according to the method of McElvain and Clarke¹ from ethyl 3,3-diethoxypropionate, which was conveniently prepared from ethyl orthoformate and ethyl bromoacetate according to Tschitschibabin.²

3. The intermediate condensation product does not exhibit the specific ultraviolet absorption spectrum of cytosine until after a brief treatment with dilute acid. The condensation probably proceeds *via* the open chain ureide intermediate, which upon acidification cyclizes to cytosine. This behavior is similar to the cyclization of cyanoacetylurée to form 6-amino-2,4-pyrimidinediol.^{3,4}

4. The molecular formula of cytosine sulfate is $C_4H_5N_3O \cdot H_2SO_4 \cdot H_2O$.

5. The ammonium salt was prepared by crystallization of cytosine from dilute ammonium hydroxide. Cytosine picrate was obtained by treating an aqueous solution of cytosine with saturated aqueous picric acid.

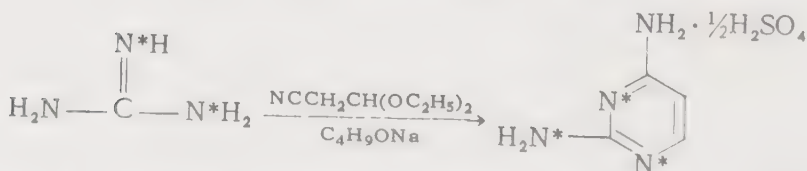
¹S. M. McElvain and R. L. Clarke, J. Am. Chem. Soc., 69, 2657 (1947).

²A. E. Tschitschibabin, J. prakt. Chem., 73, 326 (1926).

³C. K. Cain, M. F. Mallette and E. C. Taylor, Jr., J. Am. Chem. Soc., 68, 1996 (1946).

⁴F. Baum, Ber., 41, 532 (1908).

2,4-DIAMINOPYRIMIDINE-1,2,3- N_1^{15} SULFATE



A. Bendich, W. D. Geren and G. B. Brown, J. Biol. Chem., 185, 436 (1950).

A. Procedure

Guanidinium-1,2- N_1^{15} nitrate (Note 1), 2.44 g., and 3.0 ml. of cyanoacetaldehyde diethyl acetal¹ are added to 18 ml. of absolute 1-butanol.

in which 0.5 g. of sodium has been dissolved, and the mixture is refluxed for 2.5 hours with constant stirring. After the mixture is cooled, the precipitated sodium nitrate is collected, and the filtrate is acidified with sufficient 6 *N* sulfuric acid to cause precipitation of the crude, pyrimidine, 2.94 g.

The product is crystallized from 2 *N* sulfuric acid and dried at 110° over phosphorus pentoxide *in vacuo* (Note 2).

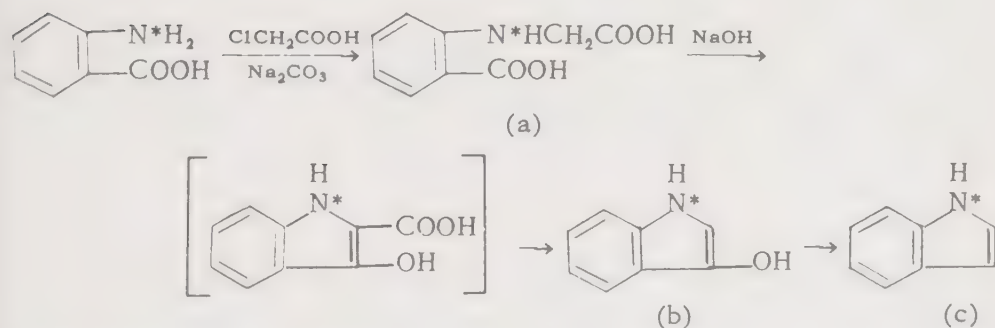
B. Notes

1. The guanidinium-1,2- $N^{15}_{1/2}$ nitrate is prepared from cyanoguanidine and ammonium- N^{15} nitrate; therefore, due to tautomerism, the product is a mixture of singly labeled molecules.

2. The 2,4-diaminopyrimidine sulfate is a hydrate; the molecular formula is $(C_4H_6N_4)_2 \cdot H_2SO_4 \cdot H_2O$.

¹A. Bendich, H. Getler and G. B. Brown, *J. Biol. Chem.*, 177, 565 (1949).

INDOLE- N^{15}



R. W. Schayer, *J. Biol. Chem.*, 187, 777 (1950).

A. Procedure

(a) *N*-Carboxymethylanthranilic- N^{15} Acid. Anthranilic- N^{15} acid is reacted with chloroacetic acid according to an adaptation of the following procedure described by Haller¹ (Note 1).

To a mixture of 25 g. of anthranilic acid and 150 ml. of water in a 1-l. flask, equipped with a reflux condenser and mercury-sealed stirrer, is added 8.6 g. of chloroacetic acid in 50 ml. of water and 22.5 g. of sodium carbonate. With stirring the mixture is heated to 90° for 1 hour. The heating bath is removed; with continued stirring, the reaction mixture is cooled and then acidified with hydrochloric acid. After 24 hours, the precipitate is collected, washed with water and dried at 100°. The yield of crude product, m.p. 207°, is 12.2 g. (68.6%, based on chloro-

acetic acid) (Note 2). To recover the unreacted anthranilic acid, 70 g. of sodium acetate is dissolved in the mother liquor. After the solution is left overnight, the precipitate of anthranilic acid is collected, washed with water and dried (Note 3). To the filtrate is added 10 g. of sodium acetate and excess saturated copper acetate solution. After 5-6 hours the precipitate of copper anthranilate is collected, washed with water and dried.

(b) *Indoxyl-N¹⁵*, (*3-Hydroxyindole-N¹⁵*). *N*-Carboxymethylantranilic-*N¹⁵* acid is converted to indoxyl-*N¹⁵* by adapting the following procedure.²

A mixture of 10 g. of *N*-carboxymethylantranilic acid, 30 g. of sodium hydroxide pellets and 10 ml. of water in a nickel or copper container is stirred with a thermometer protected by a copper tube and heated to 200-210° under an inert atmosphere. The mixture fuses and gradually assumes an orange color. The melt is cooled and dissolved in 200 ml. of water while still under an inert atmosphere (Note 4).

(c) *Indole-N¹⁵*, (*Benzopyrrole-N¹⁵*). Indoxyl-*N¹⁵* is converted to indole-*N¹⁵* by application of the procedure of Vorländer.³

The above alkaline solution of indoxyl is treated with sodium amalgam (Note 5) at 60-70° until a drop of the solution no longer turns blue upon exposure to air. Then the reaction mixture is saturated with carbon dioxide and steam-distilled with a stream of carbon dioxide flowing through the apparatus. Part of the indole, m.p. 52°, crystallizes from the distillate, and part remains dissolved. The latter material is isolated as the picrate, making the total yield 55% (Note 6).

B. Notes

1. Haller¹ has made a thorough study of the reaction between anthranilic acid and chloroacetic acid. He recommends the following conditions based on the study: a concentration of 25 g. of anthranilic acid in 200 ml. of water, a ratio of 2 moles of anthranilic acid to 1 mole of chloroacetic acid, 2.33 moles of sodium carbonate per mole of chloroacetic acid, a temperature of 90° and a 1-hour reaction period.

2. The yield of product, based on anthranilic acid used, was only 34.3%, but the yield based on anthranilic acid consumed was 83.3%. In a similar procedure described by Vogel,² but employing a 1:1 ratio of reactants, the yield of *N*-carboxymethylantranilic acid was 61.5%.

3. The recovery of unchanged anthranilic acid by this procedure is 85%.

4. According to Vorländer,⁴ indoxyl was precipitated as an oil when its solution in alkali was saturated with carbon dioxide. The oil soon solidified to a partially crystalline mass, at room temperature. The indoxyl was then dissolved in warm water, under an atmosphere of illuminating gas, filtered free of undissolved resin and cooled with an

ice-bath. The yellow crystals of indoxyl, m.p. 85° , were soluble in water, alcohol, ether, chloroform, benzene and acetic acid, easily soluble in acetone and slightly soluble in petroleum ether.

5. Baeyer⁵ reduced indoxyl to indole by distillation from zinc dust. After the oily product solidified it was recrystallized from water, m.p. 52° .

6. According to an isolation procedure described by Tyson,⁶ indole in the steam-distillate is extracted into ether and dried over sodium sulfate. After removal of ether, the indole distills at $142\text{--}144^{\circ}$ (27 mm.) (128° /10 mm.; 121° /5 mm.) as a pale-yellow oil which solidifies; m.p. $52\text{--}53^{\circ}$.

¹H. L. Haller, *Ind. Eng. Chem.*, 14, 1040 (1922).

²A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., New York, 1948, p. 949.

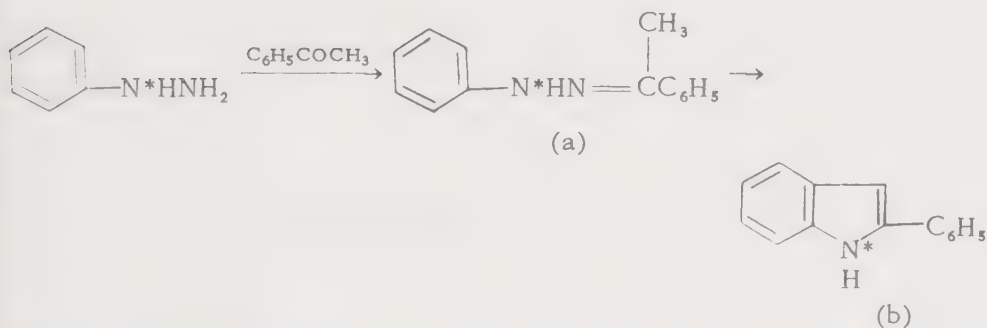
³D. Vorländer and O. Apelt, *Ber.*, 37, 1134 (1904).

⁴D. Vorländer and B. Drescher, *Ber.*, 35, 1701 (1902).

⁵A. von Baeyer, *Ann.*, Suppl., 7, 56 (1871).

⁶*Organic Syntheses*, Vol. 23, Wiley, New York, 1943, p. 43.

2-PHENYLINDOLE- N^{15}



C. F. H. Allen and C. V. Wilson, *J. Am. Chem. Soc.*, 65, 611 (1943).

A. Procedure (Note 1)

(a) 1-(α -Methylbenzylidene)-2-phenylhydrazine-2- N^{15} , (Acetophenone Phenylhydrazone-1- N^{15}). According to the following procedure of Fischer,¹ 7.4 g. of acetophenone is dissolved in alcohol, and water is added slowly, with stirring, until the cloudiness just disappears (Note 2). To the clear solution is added 7.8 g. of phenylhydrazine hydrochloride and 5.3 g. of sodium acetate. After the reagents are thoroughly mixed, the solution is gently warmed for a few minutes and then set aside to cool. The acetophenone phenylhydrazone is collected, washed with water and recrystallized from alcohol, m.p. 105° .

(b) 2-Phenylindole- N^{15} . Acetophenone 1-phenylhydrazone-1- N^{15} is converted to 2-phenylindole- N^{15} by the Fischer synthesis.^{2,3} The phenyl-

hydrazone is heated with a five-fold amount of zinc chloride for 3-5 minutes in an oil-bath at 170-180°. Upon treating the red-brown melt with very dilute hydrochloric acid, 2-phenylindole remains as a dark-colored crystalline mass. After drying on a water-bath, the material is distilled and then recrystallized from alcohol or chloroform, m.p. 186° (uncor.) (Note 3).

B. Notes

1. The authors were primarily interested in the mechanism of the Fischer indole synthesis, and few experimental details were given. Various proposed mechanisms for the reaction have been summarized by Van Order and Lindwall.⁴ The authors have interpreted the mechanism presented by Robinson⁵ in a slightly different way.

2. If too much water is added, a little alcohol is added to clarify the solution.

3. Improved procedures for the preparation of acetophenone phenylhydrazone and 2-phenylindole have been described.⁶

¹E. Fischer, Ber., 17, 572 (1884).

²E. Fischer, and O. Hess, Ber., 17, 559 (1884).

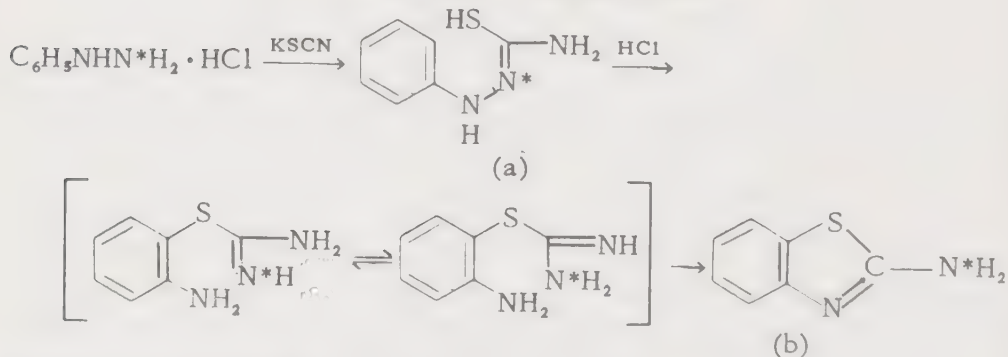
³E. Fischer, Ann., 236, 133 (1886).

⁴R. B. Van Order and H. G. Lindwall, Chem. Rev., 30, 80 (1942).

⁵G. M. Robinson and R. Robinson, J. Chem. Soc., 113, 639 (1918); *ibid.*, 125, 827 (1924).

⁶*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 98.

2-AMINOBENZOTHAZOLE-2-N¹⁵



K. Clusius and H. R. Weissner, *Helv. Chim. Acta*, 35, 400 (1952).

A. Procedure

(a) *1-Phenylthiosemicarbazide-2-N¹⁵*. 1-Phenylhydrazine-2-N¹⁵ hydrochloride, 0.85 g., is heated under reflux with 1 g. of potassium thiocyanate (Note 1) in 3 ml. of absolute alcohol for 12 hours on a water-bath.

After crystallizing overnight, the crystalline mass is collected, washed with water to remove potassium chloride, and recrystallized from alcohol. The yield of 1-phenylthiosemicarbazide-2-N¹⁵ is 0.3 g., m.p. 200–201°.

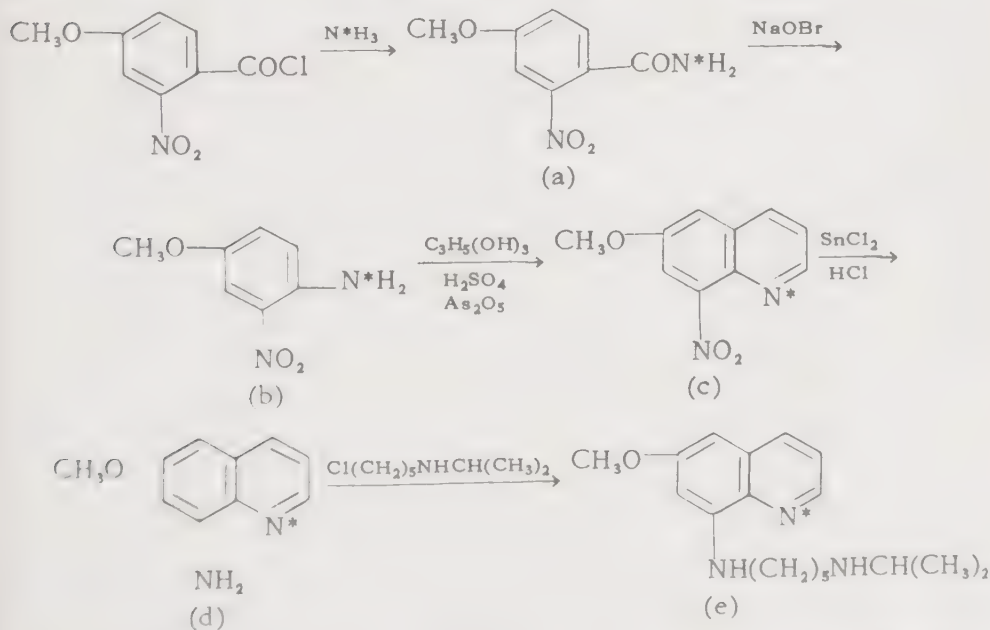
(b) 2-Aminobenzothiazole-2-N¹⁵. 1-Phenylthiosemicarbazide-2-N¹⁵, 0.3 g., is heated with 1.5 ml. of 6 *N* hydrochloric acid for 10 hours in a sealed tube at 125–130°. Upon cooling the solution, 2-aminobenzothiazole-2-N¹⁵ hydrochloride precipitates (Note 2). It is collected and dissolved in a little water, decolorized with carbon and again precipitated by saturating the solution with hydrogen chloride. The hydrochloride salt is treated with potassium hydroxide, and the precipitated free base is recrystallized from hot water and dried, m.p. 129°.

B. Notes

1. Fischer and Besthorn¹ used ammonium thiocyanate in this reaction.
2. A large part of the remaining 2-aminobenzothiazole-2-N¹⁵ is obtained if the mother liquor is made weakly basic with potassium hydroxide, since the free base is slightly soluble in cold water.

¹E. Fischer and E. Besthorn, *Ann.*, 212, 325 (1882).

8-(5-ISOPROPYLAMINOPENTYLAMINO)-6-METHOXYQUINOLINE-N¹⁵ (N¹⁵-Pentaquine)



R. C. Elderfield, L. L. Smith and E. Werble, *J. Am. Chem. Soc.*, 75, 1245 (1953).

A. Procedure

(a) *4-Methoxy-2-nitrobenzamide-N¹⁵*. 2-Nitroanisoyl chloride (Note 1) is treated with anhydrous ammonia-N¹⁵ to obtain the amide. After recrystallization from aqueous alcohol, the product melts at 160–162°.

(b) *4-Methoxy-2-nitroaniline-N¹⁵*. By the Hofmann degradation¹ of 4-methoxy-2-nitrobenzamide-N¹⁵, with sodium hypobromite, 4-methoxy-2-nitroaniline-N¹⁵ is prepared in 66% yield. The following procedure is according to Beckmann and Correns.² A solution of 10.2 g. of potassium hydroxide and 10.8 g. of bromine in 100 ml. of water is added to 0.060 mole of the amide. This mixture is then added to a solution of 14.4 g. of potassium hydroxide in 25 ml. of water. The temperature of the mixture is maintained at 70–75° for about 45 minutes. Finally the amine is distilled with steam or extracted with ether.

(c) *6-Methoxy-8-nitroquinoline-1-N¹⁵*. By using the Skraup reaction according to the procedure described by Mosher, Yanko and Whitmore,³ 4-methoxy-2-nitroaniline-N¹⁵ is converted to 6-methoxy-8-nitroquinoline-1-N¹⁵ (Note 2).

(d) *8-Amino-6-methoxyquinoline-1-N¹⁵*. 6-Methoxy-8-nitroquinoline-1-N¹⁵ is reduced with stannous chloride in concentrated hydrochloric acid according to Blatt⁴ who reports a 77% yield of the 8-amino compound (Note 3).

(e) *8-(5-Isopropylaminopentylamino)-6-methoxyquinoline-N¹⁵*, (*N₁¹⁵-Pentaquine*). N₁¹⁵-Pentaquine is synthesized according to the following procedure described by Drake.⁵ In a 250-ml. flask, equipped with a mechanical stirrer, a reflux condenser and a thermometer extending into the reaction mixture, is placed 69.6 g. (0.40 mole) of 8-amino-6-methoxyquinoline, 40.0 g. (0.20 mole) of 1-chloro-5-isopropylaminopentane hydrochloride (Note 4) and 50 ml. of water. The mixture is stirred and heated at 80° for 20 hours and then at 103° for 4 hours (Note 5). The mixture is poured into 200 ml. of water, enough concentrated sodium hydroxide solution is added to make the pH of the mixture about 4.5, and solid sodium acetate trihydrate is then added until the pH is 5.0. To recover excess 8-amino-6-methoxyquinoline, the mixture is warmed to 65° and extracted at this temperature with four 200-ml. portions of benzene (Note 6). The combined benzene extracts are washed with 20 ml. of hot water which is added to the main aqueous solution. Upon cooling the aqueous solution, the monohydrochloride of the product separates. The light-gray solid is collected, pressed dry on the filter and then dissolved in 200 ml. of water at 50°. A solution of 10 g. of sodium hydroxide in 20 ml. of water is added; the mixture is cooled to 25° and extracted with four 100-ml. portions of ether (Note 7). The combined ether extracts are washed with three 50-ml. portions of water and dried over anhydrous magnesium sulfate. After filtration and removal of ether on a steam-bath, the residue weighs 59 g. (Note 8).

The free base is dissolved in 550 ml. of 95% ethanol in a flask equipped with a stirrer, a reflux condenser and a funnel for addition of acid. With stirring the mixture is refluxed while a solution of 17 g. (0.147 mole) of 85% phosphoric acid in 30 ml. of 95% ethanol is added over a period of five minutes. Pale-yellow crystals of the monophosphate soon appear; the mixture is refluxed for 15 minutes and then allowed to cool with stirring for 1 hour. The mixture is then cooled in an ice-bath for 2 hours with stirring. The product is collected, washed with 50 ml. of ice-cold 95% ethanol and finally dried in a vacuum oven at 50°. The yield of monophosphate, m.p. 189–189.5°, is 56.0 g. (70%) (Note 9).

B. Notes

1. In the preparation of 4-methoxy-2-nitrobenzoic acid, *p*-toluidine was nitrated according to Nolting and Collin⁶ to obtain 3-nitro-*p*-toluidine in 65% yield. The latter compound was diazotized to obtain 3-nitro-*p*-cresol⁷ in 36% yield. Methylation of 3-nitro-*p*-cresol with methyl sulfate, according to Harvey,⁷ gave 4-methyl-3-nitroanisole in 88% yield. The latter compound was oxidized to 2-nitro-*p*-anisic acid with permanganate according to Ullmann.⁸

2. 6-Methoxy-8-nitroquinoline, m.p. 158–160°, was obtained in 65–76% yield.³

3. The reduction of 6-methoxy-8-nitroquinoline to 8-amino-6-methoxyquinoline, b.p. 15° (1 mm.), m.p. 53°, in 95% yield, using a 5% palladium-on-barium sulfate catalyst, was reported by Haskelberg.⁹

4. The synthesis of 1-chloro-5-isopropylaminopentane hydrochloride is described by Drake.⁵

5. These temperatures are of the reaction mixture.

6. From the dried, combined benzene extracts was obtained 35 g. of the starting material.

7. A small amount of black emulsion appeared between the layers during the first extraction; the emulsion layer was removed and filtered with vacuum, and the filtrate was returned to the aqueous phase for further extraction. A thin layer of black tar remained on the filter.

8. A small aliquot of the residue was dissolved in excess 0.1 *N* hydrochloric acid and back-titrated to about pH 6.8 with standard alkali; from this titration the amount of base in the residue was calculated.

9. The free base, 8-(5-isopropylaminopentylamino)-6-methoxyquinoline, boils at 165–170° at 20 microns (bath temperature, 200–210°); n_D^{25} 1.5785. The monohydrochloride melts at 152–153°. Its solubility in water at 10° is 0.012 g./ml.; in ethanol, 0.026 g./ml. at 5°. The dihydrochloride decomposes at 218–219° with previous sintering at 216°. Its solubility in water at 10° is 0.5 g./ml.; in ethanol, 0.017 g./ml. at 5°.

¹*Organic Reactions*, Vol. III, Wiley, New York, 1946, p. 267.

²E. Beckmann and E. Correns, *Ber.*, 55, 850 (1922).

³*Organic Syntheses*, Vol. 27, Wiley, New York, 1947, p. 48.

⁴A. H. Blatt and N. Gross, *J. Am. Chem. Soc.*, 75, 1245 (1953).

⁵N. L. Drake, J. Van Hook, J. A. Garman, R. Hayes, R. Johnson, G. W. Kelley, S. Melamed and R. M. Peck, *ibid.*, 68, 1529, (1946).

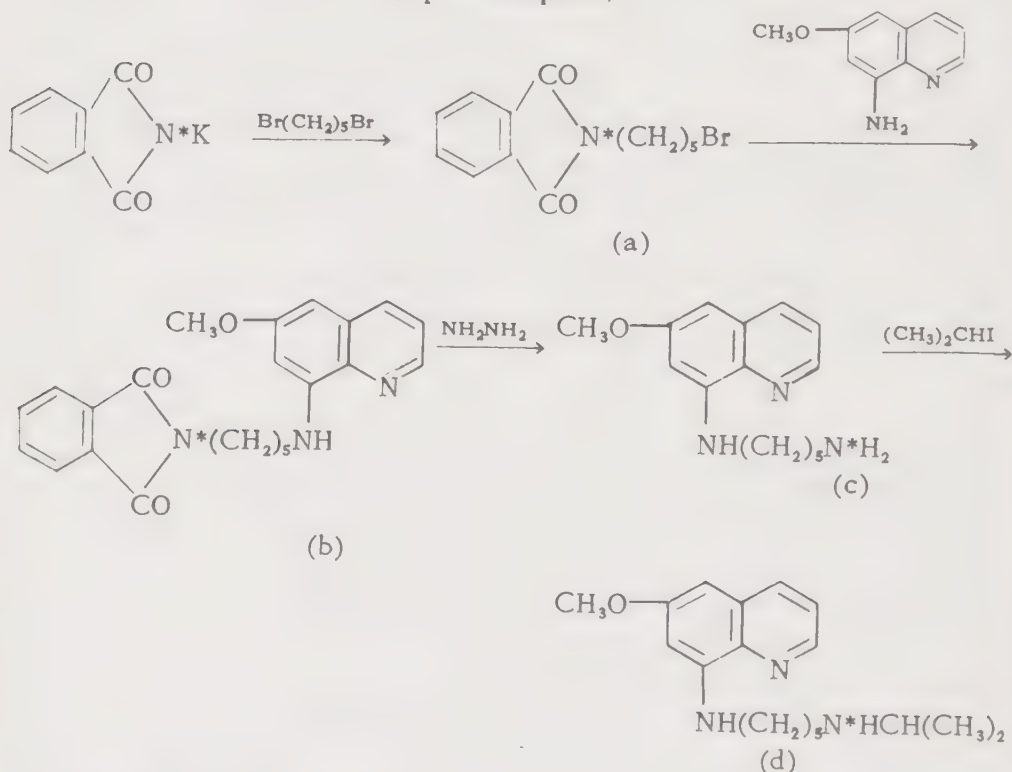
⁶E. Nolting and A. Collin, *Ber.*, 17, 261 (1884).

⁷D. G. Harvey and W. Robson, *J. Chem. Soc.*, 1938, 97.

⁸F. Ullmann and P. Dootson, *Ber.*, 51, 9 (1918).

⁹L. Haskelberg, *J. Org. Chem.*, 12, 434 (1947).

8-(5-ISOPROPYLAMINO-N¹⁵-PENTYLAMINO)-6-METHOXYQUINOLINE (N¹⁵-Pentaquine)



A. H. Blatt and N. Gross, *J. Am. Chem. Soc.*, 75, 1245 (1953).

A. Procedure

(a) *N*-(5-Bromopentyl)phthalimide-N¹⁵.¹ A mixture of 105.0 g. (0.65 mole) of potassium phthalimide-N¹⁵ and 128.5 g. (0.56 mole) of pentamethylene bromide in 500 ml. of acetone is stirred and boiled under reflux for 48 hours. The bulk of the acetone is removed by distillation, and the residual solid-liquid mixture is filtered to remove *N,N'*-pentamethylenebis(phthalimide-N¹⁵), potassium bromide, and unreacted potassium phthalimide-N¹⁵. Most of the acetone and unreacted pentamethylene bromide in the filtrate are removed by distillation, and the residue is filtered to

remove a second crop of *N,N'*-pentamethylenebis(phthalimide- N^{15}). The addition of 30 ml. of ethanol to the second filtrate furnishes 88.0 g. (52.5%) of *N*-(5-bromopentyl)phthalimide- N^{15} , m.p. 56–58°. The by-product, *N,N'*-pentamethylenebis(phthalimide- N^{15}), when purified weighs 19.0 g.

(b) *6-Methoxy-8-(5-phthalimido- N^{15} -pentylamino)quinoline*.¹ A mixture of 88.0 g. (0.3 mole) of *N*-(5-bromopentyl)phthalimide- N^{15} and 103.5 g. (0.59 mole) of twice-distilled 8-amino-6-methoxyquinoline is stirred and heated to 125°. The mixture is maintained at this temperature for one-half hour, then heated on the steam-bath for 1 hour, after which 450 ml. of ethanol is added, and the mixture is refluxed for 22.5 hours. The hot reaction mixture is transferred to a Waring Blendor, cooled to room temperature, and slurried. The precipitate is filtered, washed with ethanol, and treated with 50 ml. of saturated potassium carbonate solution. The liberated base is extracted with 100 ml. of chloroform, and the chloroform extract is washed with water and dried over anhydrous sodium sulfate. The chloroform is removed by distillation, and the residue is crystallized from 300 ml. of ethanol to obtain 79.0 g. (68%) of the phthalimido- N^{15} -quinoline as light tan crystals melting at 113–115°.

(c) *8-(5-Amino- N^{15} -pentylamino)-6-methoxyquinoline Dihydrochloride*.¹ To a suspension of 79.0 g. (0.2 mole) of 6-methoxy-8-(5-phthalimido- N^{15} -pentylamino)quinoline in 600 ml. of ethanol, 13.4 ml. (0.23 mole) of 85% hydrazine hydrate is added. The reaction mixture is stirred and refluxed for 2 hours during which time the solid dissolves. A total of 350 ml. of ethanol is removed by distillation, and the residue is cooled and filtered. The filtrate is concentrated under reduced pressure, and the residual solid is combined with the first precipitate. The combined solids are heated under reflux with a boiling mixture of 175 ml. of ethanol and 67.3 ml. of concentrated hydrochloric acid. The reaction mixture is then cooled and filtered from phthalhydrazide. The filtrate is concentrated under reduced pressure, and the residue is crystallized from 125 ml. of ethanol to give 69.0 g., a quantitative yield, of 8-(5-amino- N^{15} -pentylamino)-6-methoxyquinoline dihydrochloride, m.p. 152–158° (Note 2).

(d) *8-(5-Isopropylamino- N^{15} -pentylamino)-6-methoxyquinoline*, (N_1^{15} -Pentaquine) (Note 2). The 69.0 g. of dihydrochloride obtained in the preceding preparation is dissolved in 350 ml. of water, and 95.0 g. (0.9 mole) of sodium carbonate is added with stirring. Next, 40 ml. (0.4 mole) of isopropyl iodide is added, and the mixture is refluxed for 4 hours, cooled, and extracted with 50 ml. of ether. A violet solid, m.p. 164–167°, which precipitates on addition of the ether, is removed by filtration and dissolved in 200 ml. of ethanol. The ethanol solution is heated to boiling, and 6.4 ml. of 85% phosphoric acid is added dropwise with stirring. A solid begins to precipitate during the addition. The solution is boiled under reflux for 15 minutes after the addition is com-

plete, and stirring is continued while the solution cools to room temperature. The solution is left overnight in a refrigerator, then filtered to obtain 26 g. of crude product, m.p. 184-187°. Crystallization from 135 ml. of ethanol and 35 ml. of water furnishes 22.5 g. (27%) of N_1^{15} -pentaquine monophosphate, m.p. 188-189° (Note 3).

B. Notes

1. The over-all yield in the first three reactions was 36%, compared with 39% obtained by Drake,¹ whose directions were used.

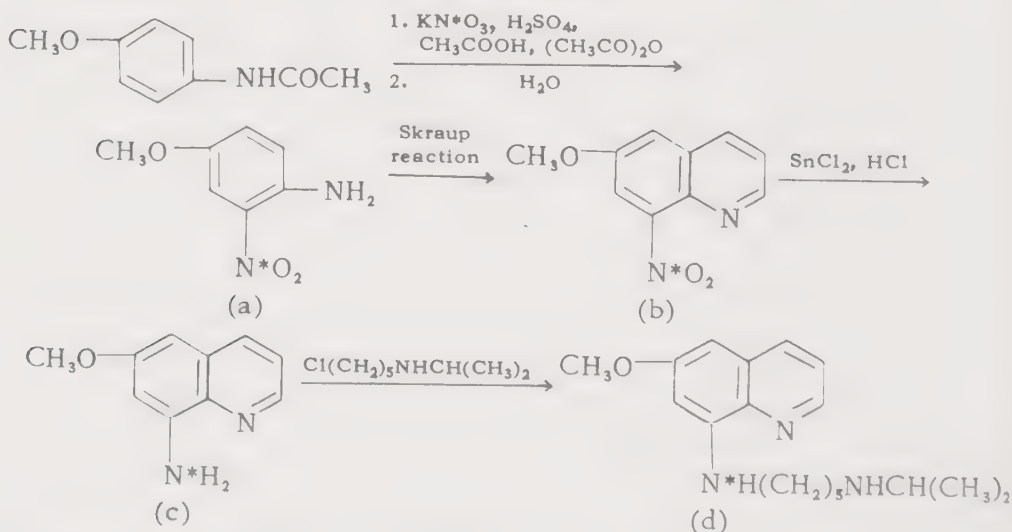
2. The final reaction, the attachment of the isopropyl group, had not been done before. Reductive alkylation with acetone in preliminary experiments with non-labeled material gave erratic results. Therefore, direct alkylation with isopropyl iodide was used, following a procedure developed by Baldwin² for the corresponding alkylation with butyl iodide.

3. The N_1^{15} -pentaquine obtained as the monophosphate contained 20.5 atom per cent excess N^{15} and had a 98% homogeneity by countercurrent extraction.

¹Prepared according to a private communication from N. L. Drake to Blatt and Gross.

²A. W. Baldwin, J. Chem. Soc., 1929, 2959.

8-(5-ISOPROPYLAMINOPENTYLAMINO- N^{15})-6-METHOXYQUINOLINE (N_1^{15} -Pentaquine)



A. H. Blatt and N. Gross, J. Am. Chem. Soc., 75, 1245 (1953).

A. Procedure

(a) 4-Methoxy-2-nitroaniline-2- N^{15} (Note 1). To a solution of 127.5 g. (0.77 mole) of recrystallized *p*-acetanisidide in 485 ml. of warm glacial

acetic acid is added 485 ml. of acetic anhydride. A nitrating mixture is prepared by dissolving 85 g. (0.84 mole, 1.09 equivalents) of potassium nitrate- N^{15} in 90 ml. of hot water and adding a cooled solution of 46 ml. of concentrated sulfuric acid in 74 ml. of water. The nitrating mixture is added dropwise during 1.5 hours to the solution of *p*-acetanisidide which has been cooled to 10–12°, and which is held at this temperature by external cooling during the reaction. The dropping funnel is rinsed with 10 ml. of glacial acetic acid which is added to the reaction mixture. The originally colorless solution of *p*-acetanisidide turns a deep yellow, and a large amount of solid precipitates shortly after the addition is begun. Toward the end of the addition, the solution is deep red and most of the solid redissolves.

The reaction mixture is stirred for 2 hours at 10–12° after the addition is complete. It is then allowed to warm to room temperature and is poured onto 1 l. of ice and water. The bright yellow precipitate is filtered and washed with three 75-ml. portions of water. It is then hydrolyzed without drying, by heating it under reflux for 3 hours with 290 ml. of methanol and 96 ml. of concentrated hydrochloric acid. During the hydrolysis, the solid dissolves giving a clear red solution.

The acidic solution is cooled and poured onto 580 g. of ice and 200 ml. of concentrated ammonium hydroxide. The bright red precipitate of 4-methoxy-2-nitroaniline-2- N^{15} is collected, washed with three 100 ml. portions of water, and dried; yield 104.5 g., m.p. 117–117.5°. The product is crystallized by dissolving it in 1500 ml. of hot methanol, concentrating the solution to about 500 ml., and chilling. The recrystallized material melts sharply at 118° (literature, 124°) and weighs 100 g. The yield is 77% based on *p*-acetanisidide, 70% based on potassium nitrate- N^{15} .

(b) 6-Methoxy-8-nitroquinoline-8- N^{15} .² The 4-methoxy-2-nitroaniline-2- N^{15} is converted to 6-methoxy-8-nitro- N^{15} -quinoline in 65% yield by the Skraup reaction.

(c) 8-Amino-6-methoxyquinoline-8- N^{15} .³ The 79 g. (0.38 mole) of 6-methoxy-8-nitroquinoline-8- N^{15} thus obtained is dissolved in 285 ml. of warm (50–60°) concentrated hydrochloric acid, and the solution is added dropwise, with stirring, to a slurry of 284 g. (1.26 moles) of stannous chloride dihydrate and 95 ml. of concentrated hydrochloric acid. During the addition, which requires one-half hour, the reaction mixture is held at 40–50° by external cooling. The product precipitates as yellow crystals. Fifteen minutes after the addition is complete, the reaction mixture is cooled to 20° in an ice-bath and 556 ml. of saturated aqueous potassium hydroxide is added dropwise, at such a rate that the temperature does not exceed 40°. Stirring is continued for an additional hour.

The alkaline solution and suspended solid are extracted with one 200-ml. portion and two 100-ml. portions of ether. The combined ether extracts are washed with three 100-ml. portions of water and dried over

calcium chloride. After removal of ether, the residue is distilled at 162° (4 mm.), and the distillate is immediately redistilled to yield 44 g. of 8-amino- N^{15} -6-methoxyquinoline, b.p. $143\text{--}144^{\circ}$ (2 mm.) (Note 2).

(d) 8-(5-Isopropylaminopentylamino- N^{15})-6-methoxyquinoline, (N_1^{15} -Pentaquine). A mixture of 44 g. (0.25 mole) of 8-amino-6-methoxyquinoline- N^{15} , 25 g. (0.125 mole) of 1-chloro-5-isopropylaminopentane hydrochloride, and 32 ml. of water is heated at 80° for 20 hours with stirring. The temperature is then raised to 103° for 4 hours, after which the melt is poured into 120 ml. of water. The pH of the mixture is 3.85. The addition of 7 ml. of 30% aqueous sodium hydroxide followed by 8.5 g. of sodium acetate raises the pH to the desired value of 5. The mixture is then warmed to 65° and extracted with four 125-ml. portions of benzene. The combined benzene layers are washed with 20 ml. of hot water, which is added to the principal aqueous layer (Note 3).

The warm aqueous layer is allowed to cool, and the precipitate that forms is separated by filtration and dissolved in 120 ml. of water at 50° . The solution is neutralized by the addition of a solution of 6 g. of sodium hydroxide in 12 ml. of water, cooled to 25° , and extracted with four 125-ml. portions of ether. The combined ether extracts are washed with three 30-ml. portions of water, dried over anhydrous potassium carbonate and concentrated to a volume of 50 ml. To this ethereal solution is added 230 ml. of ethanol. The alcohol-ether solution is heated to boiling, and 10.2 g. of 85% phosphoric acid in 30 ml. of ethanol is added dropwise with stirring. A voluminous tan precipitate forms during the addition. The solution is boiled under reflux for 15 minutes after the addition is complete, and stirring is continued while the solution cools to room temperature. The solution is left overnight in a refrigerator, then filtered to yield 32 g. (64%) of N_1^{15} -pentaquine monophosphate, m.p. $188.5\text{--}190^{\circ}$ (Note 4).

B. Notes

1. The most satisfactory procedure for this nitration¹ uses an excess (1.6 equivalents) of 70% nitric acid since this is less expensive than *p*-acetanisidide. Concentrated nitric acid enriched in N^{15} was not available, so the easily handled potassium nitrate- N^{15} was used rather than to concentrate dilute nitric- N^{15} acid. Conditions for the most efficient use of the nitrating agent were worked out with unlabeled materials. The results, because of their possible general interest, are given in brief form.

- (a) Potassium nitrate and acetic acid will not nitrate *p*-acetanisidide; a mineral acid is essential. Sulfuric acid is the obvious choice and it was used in all subsequent experiments.

- (b) Sulfuric acid cannot be used as the solvent for the nitration, for in this solvent the nitro group takes the position *ortho* to the methoxyl group. Acetic acid was therefore used as the solvent.
- (c) The nitrating agent (potassium nitrate and sulfuric acid) must be added to the solution of *p*-acetanisidide in acetic acid. Addition of *p*-acetanisidide in acetic acid to the nitrating agent results in the introduction of the nitro group in the *ortho* position to the methoxyl group.
- (d) A mixture of potassium nitrate, sulfuric acid, and sufficient water to approximate 70% nitric acid is a satisfactory nitrating agent. However, some of the potassium nitrate does not dissolve, and the loss would be prohibitively wasteful and expensive with potassium nitrate containing N^{15} .
- (e) A mixture of potassium nitrate and sulfuric acid, to which sufficient water has been added to dissolve the salt, is satisfactory for nitration if the *p*-acetanisidide is dissolved in acetic acid plus enough acetic anhydride to convert the added water to acetic acid.
- (f) It is more economical to use a slight excess of nitrating agent than to take the equivalent amount and then separate unreacted *p*-acetanisidide from the nitration product.

With the information from these experiments a procedure was evolved for nitrating *p*-acetanisidide in acetic acid-acetic anhydride solution with a solution of potassium nitrate, sulfuric acid and water. The procedure gave satisfactory results, as a comparison with the results of Mosher, *et al.*¹ shows.

	Equivalents of nitrate ion	Yield	
		Based on <i>p</i> - acetanisidide	Based on nitrate ion
Mosher, Yanko, Whitmore	1.6	85%	57%
This work	1.09	77%	70%

2. The residue from the first distillate is left in the distilling flask, and the flask is used to distill the 8-amino-6-methoxyquinoline-8- N^{15} recovered in the preparation of N_1^{15} -pentaquine. The amount of 8-amino-6-methoxyquinoline-8- N^{15} recovered in this way was 7 g. more than the theoretical quantity, so that the yield in the reduction of the nitroquinoline amounted to 51 g. or 77%.

3. From the benzene, the excess 8-amino-6-methoxyquinoline-8- N^{15} was recovered by drying, removing the benzene by distillation, and distilling the residue under reduced pressure. In this way, using the flask in which the crude aminoquinoline had been first distilled (see Note 2), the recovery of twice-distilled 8-amino-6-methoxyquinoline-8- N^{15} (b.p. 172-

174°/7 mm., m.p. 40-42°) was 29 g., or 7 g. more than the theoretical amount.

4. The over-all yield was 22.5%, as compared with an over-all yield of 24.7% for the same reactions as reported in the literature.

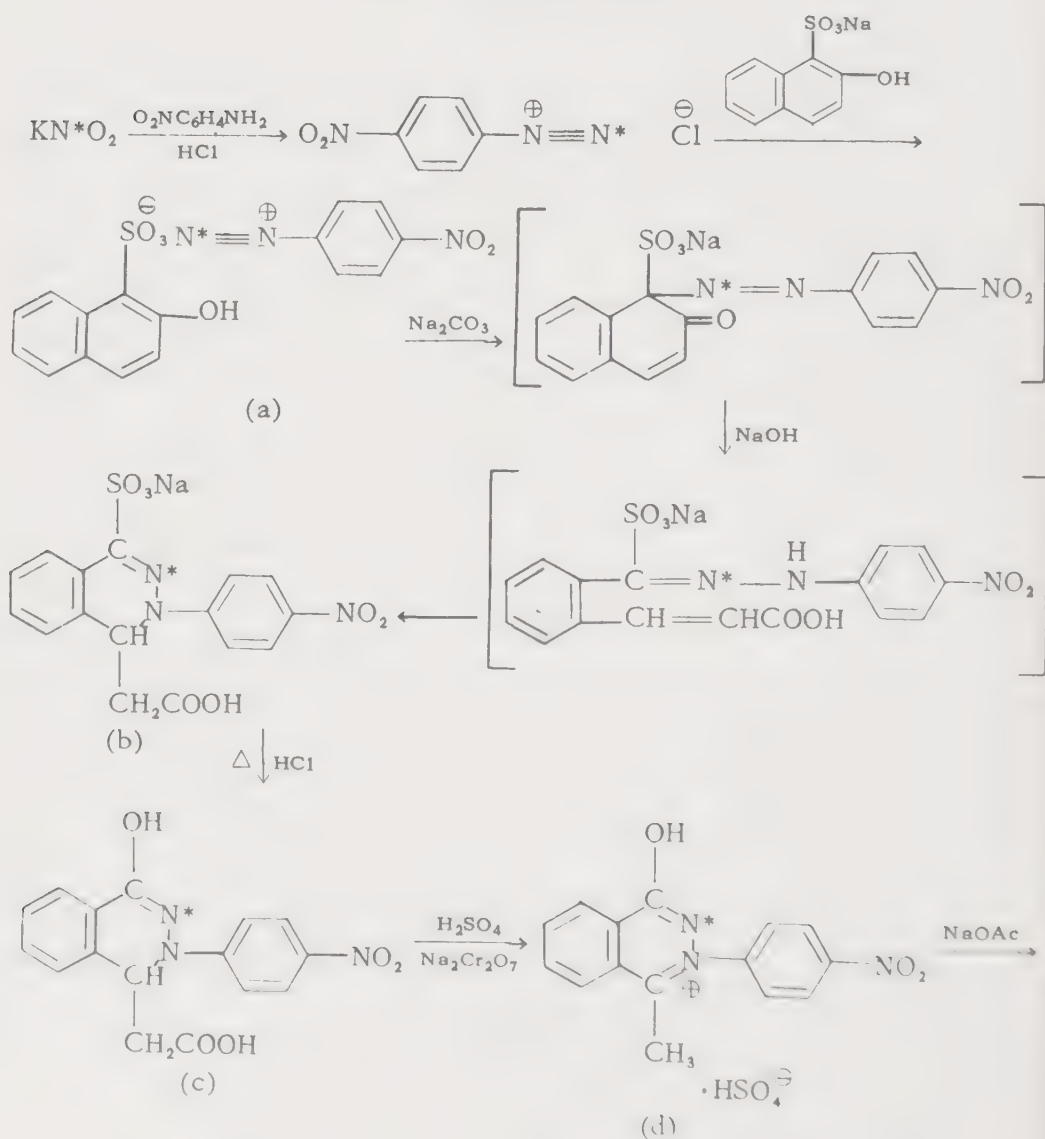
¹Directions of H. S. Mosher, W. H. Yanko and F. C. Whitmore for the nitration made available by R. C. Elderfield.

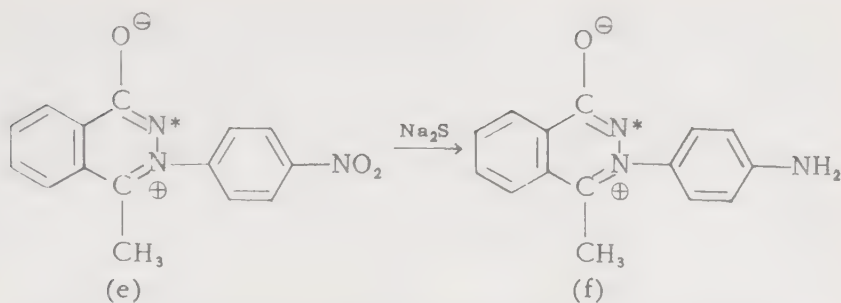
²H. S. Mosher, W. H. Yanko and F. C. Whitmore, *Organic Syntheses*, 27, 48 (1947).

³Directions of T. A. Williamson, made available by R. C. Elderfield.

⁴N. L. Drake, J. Van Hook, J. A. Garman, R. Hayes, R. Johnson, G. W. Kelley, S. Melamed and R. M. Peck, *J. Am. Chem. Soc.*, 68, 1529 (1946).

2-(4-AMINOPHENYL)-4-HYDROXY-1-METHYLPHTHALAZINIUM-3-N¹⁵ HYDROXIDE INNER SALT





W. R. Vaughan, D. I. McCane and G. J. Sloan, J. Am. Chem. Soc., 73, 2298 (1951).

A. Procedure (Note 1)

(a) *4-Nitrobenzenediazonium- β -N¹⁵ 2-Naphthol-1-sulfonate*. In 60 ml. of concentrated hydrochloric acid and 240 ml. of water, 24 g. of *p*-nitroaniline is diazotized by adding a concentrated solution of 14 g. of sodium nitrite. To the cold, stirred solution of *p*-nitrobenzenediazonium chloride is added a filtered solution of 48 g. of sodium 2-naphthol-1-sulfonate in 220 ml. of water. The 4-nitrobenzenediazonium-2-naphthol-1-sulfonate separates immediately as an orange precipitate in quantitative yield. The product is collected and washed free of acid with salt solution.

(b) *Sodium 4-Carboxymethyl-3,4-dihydro-3-(4-nitrophenyl)-1-phthalazine-sulfonate-2-N¹⁵*. The diazonium sulfonate (a) is made into a paste with 150 ml. of cold water and stirred into a cold solution of 48 g. of anhydrous sodium carbonate in 120 ml. of water (Note 2). The cold, orange solution is added immediately to a cold solution of 40 g. of sodium hydroxide in 80 ml. of water. The temperature rises about 15°, and the deep crimson mixture is left until the color changes completely to yellowish-brown. The solution is made faintly acidic with hydrochloric acid, then alkaline with sodium carbonate, and a trace of Para-red¹ is filtered off. From the filtrate, made slightly acidic with hydrochloric acid, the product separates completely as a yellow semi-crystalline precipitate. After drying, the product is extracted from sodium chloride with absolute alcohol, from which it crystallizes in orange needles; yield 66 g. (91.5%). The compound is readily soluble in water but less soluble in alcohol.

(c) *4-Carboxymethyl-3,4-dihydro-3-(4-nitrophenyl)-1-phthalazinol-2-N¹⁵*. A solution of 32 g. of the sodium sulfonate compound in 120 ml. of water containing 64 ml. of concentrated hydrochloric acid is heated under reflux until the evolution of sulfur dioxide ceases, and the product, which separates first as an oil, forms yellowish-brown crystals. The latter are washed with boiling water and recrystallized from ethyl acetate in pale yellow prisms, m.p. 241°; yield 24 g. (94%).

(d) *4-Hydroxy-1-methyl-2-(4-nitrophenyl)phthalazinium-3-N¹⁵ Sulfate*. A solution of 10 g. of 4-carboxymethyl-3,4-dihydro-3-(4-nitrophenyl)-1-phthalazinol-2-N¹⁵ in 100 ml. of cold concentrated sulfuric acid is poured on 400

g. of ice, and 4.5 g. of powdered sodium dichromate is added, in small portions, during one-half hour (Note 3). The pale-yellow precipitate in the frothy mixture is replaced gradually by a nearly colorless crystalline precipitate which is collected the next day. The product is pressed well on the filter and dried at room temperature. The crude product, 11 g., froths at 95° and melts at 231°. It dissolves readily in dry methyl or ethyl alcohol with effervescence and crystallizes in colorless plates, m.p. 246°.

(e) *4-Hydroxy-1-methyl-2-(4-nitrophenyl)phthalazinium-3-N¹⁵ Hydroxide Inner Salt*. Vaughan, *et al.*, prepared the free base by treating 5.0 g. of the phthalazinium sulfate with 20 ml. of water containing 2.5 g. of sodium acetate (Note 4). The solution is boiled, with good stirring, until all brown material becomes yellow. After cooling the mixture, 2.42 g. (65%) of the free base, m.p. 251–252° (dec.), is obtained.

(f) *2-(4-Aminophenyl)-4-hydroxy-1-methylphthalazinium-3-N¹⁵ Hydroxide Inner Salt*. The nitrophenyl inner salt, 2.3 g., is reduced in a boiling solution of 18 g. of sodium sulfide in 18 ml. of water. After 10 minutes, the reddish-orange color of the solution becomes yellow, and orange needles separate. The yield is 1.55 g. (76%), m.p. 277° (Note 5).

B. Notes

1. This series of compounds was prepared by Vaughan and co-workers with nitrogen-15 isotope, using the procedure described by Rowe, *et al.*^{2,3} Vaughan does not give experimental details but states that similar yields and identical melting points were obtained. The procedural details are taken directly from the works of Rowe, *et al.*

2. A solution, is obtained but, if it is kept, crystals of sodium 1,2-dihydro-1-(4-nitrophenylazo-1-N¹⁵)-2-oxo-1-naphthalenesulfonate separate.

3. The reaction is best carried out at 10° in a rough-walled vessel, with vigorous stirring and scratching of the walls; otherwise, the product is intractable.

4. Rowe, *et al.*,³ treated the sulfate consecutively with sodium carbonate, hydrochloric acid, and sodium carbonate.

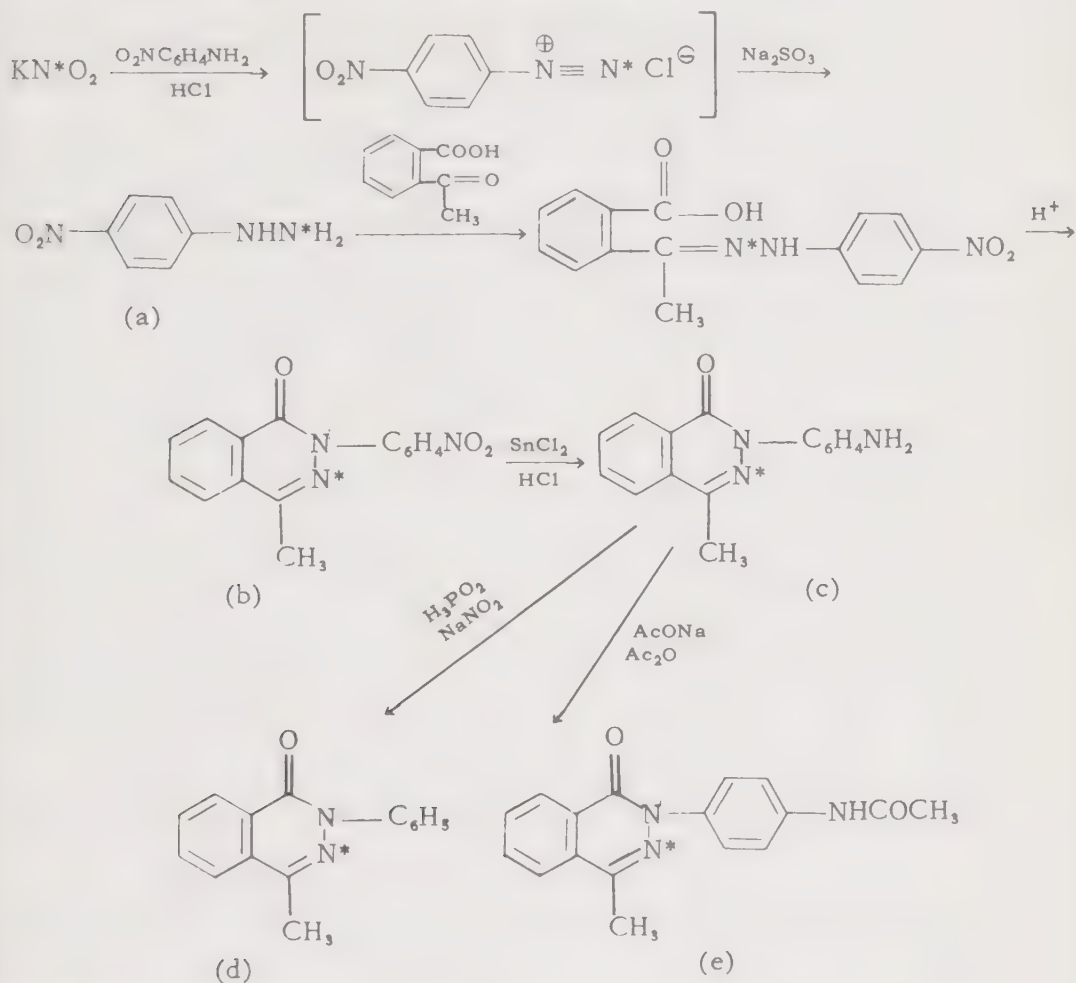
5. The readily formed acetyl derivative crystallizes from alcohol in colorless needles, m.p. 316–317°.

¹J. T. Hewitt and H. V. Mitchell, *J. Chem. Soc.*, 89, 1169 (1906).

²F. M. Rowe, E. Levin, A. C. Burns, J. S. H. Davies and W. Tepper, *ibid.* 1926, 690.

³F. M. Rowe, E. Levin, and A. T. Peters, *ibid.*, 1931, 1067.

4-METHYL-2-PHENYL-1(2*H*)-PHTHALAZINONE-3-N¹⁵
 (4-Methyl-2-phenylphthalazone-3-N¹⁵)



W. R. Vaughan, D. J. McCane and G. J. Sloan, J. Am. Chem. Soc., 73, 2298 (1951).

A. Procedure

(a) *1-(4-Nitrophenyl)hydrazine-2-N¹⁵*. This hydrazine is prepared by diazotizing 4-nitroaniline with potassium nitrite-N¹⁵ and reducing the diazonium salt with alkaline sodium sulfite,¹ as in the following example. A solution of 4-nitroaniline, 10 g., in 21 g. of concentrated hydrochloric acid, cooled to 0°, is treated with a concentrated nitrite solution. The filtered diazonium chloride solution is added with stirring, during 5 minutes, to an ice-cold solution of 41 g. of sodium sulfite in 100 ml. of water containing 4 g. of sodium hydroxide. After 5 minutes, the solution is acidified with concentrated hydrochloric acid, 70 ml., and then heated on a water-bath at 55° for 3 minutes. The mixture is kept overnight; the yellow, crystalline mass which separates is collected and

heated on a steam-bath with 20 ml. of concentrated hydrochloric acid for 7 minutes. After some time in the cold, the precipitate of crude 1-(4-nitrophenyl)hydrazine hydrochloride is collected, dissolved in water, and treated with a concentrated solution of sodium acetate. The yield of 1-(4-nitrophenyl)hydrazine which separates is about 7-8 g. after recrystallization from aqueous alcohol, m.p. 157° (dec.).

(b) 4-Methyl-2-(4-nitrophenyl)-1(2H)-phthalazinone-3-N¹⁵. 1-(4-Nitrophenyl)hydrazine-2-N¹⁵, 2.0 g., is dissolved, on a steam-bath, in a minimum of absolute ethanol. To this solution, there is added a warm solution of 4 g. of 2-acetylbenzoic acid² in 10 ml. of 95% ethanol. Without isolation of the hydrazone, the resulting solution is treated with a drop of 10% hydrochloric acid to effect cyclization. Immediately, a colorless precipitate forms, and the mixture is warmed on a steam-bath for 15 minutes. The crude product, 3.5 g. (95%), m.p. 211-212°, is obtained on filtration of the cooled reaction mixture. Recrystallization from acetic acid-water yields a product melting at 215.5-216.5°.

(c) 2-(4-Aminophenyl)-4-methyl-1(2H)-phthalazinone-3-N¹⁵. The nitrophenylphthalazone, 2.00 g., is reduced by shaking at room temperature with 10 g. of stannous chloride dihydrate in 30 ml. of concentrated hydrochloric acid until dissolution is nearly complete (Note 1). While the mixture remains on a steam-bath for 1 hour, a crystalline precipitate appears. The mixture is then cooled to 5° and poured into a cold solution of 30 g. of sodium hydroxide in 45 ml. of water which is kept at 5° with an ice-bath. The solid, collected and washed with water, is 1.79 g. (100%) of 2-(4-aminophenyl)-4-methyl-1(2H)-phthalazinone-3-N¹⁵, m.p. 211-212° (Note 2).

(d) 4-Methyl-2-phenyl-1(2H)-phthalazinone-3-N¹⁵. A mixture of 2-(4-aminophenyl)-4-methyl-1(2H)-phthalazinone-3-N¹⁵, 1.72 g., and 22.5 ml. of 25% hypophosphorous acid is treated with 0.52 g. of sodium nitrite in 2.3 ml. of water. The mixture is stirred at 5° for one-half hour and then at room temperature for 4 hours. The resulting precipitate, collected and dried in air, weighs 1.54 g. (95%), m.p. 97-98°. Recrystallized from ethanol-water, the product melts at 101.5-102.5° (Note 3).

(e) 2-(4-Acetamidophenyl)-4-methyl-1(2H)-phthalazinone-3-N¹⁵. A stirred solution of 2-(4-aminophenyl)-4-methyl-1(2H)-phthalazinone-N¹⁵, 1.72 g., in 34 ml. of boiling 3 N hydrochloric acid, is treated with 8.5 g. of anhydrous sodium acetate and 4 ml. of acetic anhydride. The yield of acetyl derivative is 2.0 g. (quantitative), m.p. 257.5-259°.

B. Notes

1. During this time, about 15 minutes, some heat is evolved.
2. Rowe³ obtained a less pure sample, m.p. 206-207°, by reduction of the nitro compound with sodium sulfide.

3. The melting point and mixture melting point of this compound were identical with that (102°) of an authentic sample prepared from 2-acetylbenzoic acid and phenylhydrazine according to Roser.⁴

C. Other Preparations

By use of the Rowe rearrangement, Vaughan, *et al.*⁵ prepared 4-methyl-2-(4-nitrophenyl)-1(2*H*)-phthalazinone-3- N^{15} , using a modification of the procedure of Rowe,⁶ from 4-methyl-3-(4-nitrophenyl)-1(2*H*)-phthalazinone-3- N^{15} sulfate. Sulfuric acid (36 ml. of 1.2 *N* acid per 2.5 g. of the phthalazine) is used in place of the hydrochloric acid of Rowe.⁶ Heating the solutions in sealed tubes at 180° for 9 hours gave 50–60% yields of the 2-(4-nitrophenyl)-4-methyl-1(2*H*)-phthalazinone; m.p. 215.5 – 216.5° , after recrystallization from acetic acid-water.

¹W. J. Hickenbottom, *Reactions of Organic Compounds*, 2nd ed., Longmans, Green and Co., London, 1948, p. 387.

²H. L. Yale, *J. Am. Chem. Soc.*, **69**, 1547 (1947).

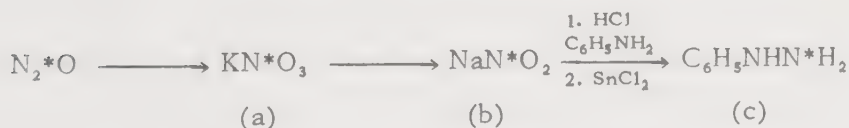
³F. M. Rowe, G. M. Heath and C. V. Patel, *J. Chem. Soc.*, 1936, 311.

⁴W. Roser, *Ber.*, **18**, 802 (1885).

⁵W. R. Vaughan, D. I. McCane, and G. J. Sloan, *J. Am. Chem. Soc.*, **73**, 2298 (1951).

⁶F. M. Rowe, D. A. W. Adams, A. T. Peters and A. E. Gillam, *J. Chem. Soc.*, 1937, 90.

1-PHENYLHYDRAZINE-2- N^{15}



K. Clusius and M. Hoch, *Helv. Chim. Acta*, **33**, 2122 (1950).

A. Procedure

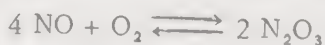
(a) *Potassium Nitrate- N^{15}* . Nitrous- N_2^{15} oxide (Note 1) is oxidized to nitric- N^{15} oxide with oxygen in a high frequency discharge. The nitric- N^{15} oxide is then oxidized quantitatively in the presence of water to nitric- N^{15} acid.

The first reaction vessel is a 100-ml. flask with two sealed-in iron electrodes, 2 mm. thick, the ends of which are 15 mm. apart. The electrodes are connected to the secondary side of a 6 KV-transformer, the primary coil of which is energized with 50-cycle current. From the top of this flask a line passes through a heating coil, and is attached to the line from the storage flasks to a cold-trap. The cold-trap is in turn connected to the bottom of the discharge flask. Between the storage

flask and the cold-trap are suitably placed stopcocks, a manometer, a vacuum line, and an oxygen inlet (Note 2). The heat of the arc and the 200° temperature of the coil around the line above the reaction flask cause a rapid convection stream from the reaction vessel through the 10-mm. tube leading back to the cold-trap. The nitric oxide, preponderantly nitric-N¹⁵ oxide with a little nitrogen-N¹⁵₂ trioxide, is frozen in the cold-trap with a Dry Ice-methanol bath. The progress of the reaction is followed by the drop in pressure indicated by the manometer. Nitrous-N¹⁵₂ oxide, from a reservoir flask, and dry oxygen, from a tank, are brought together in the reactor; after a short time the presence or absence of a brown-red color in the recycle line indicates which gas is to be increased. When all the nitrous-N¹⁵₂ oxide has been transferred from the reservoir, the working pressure of 600 mm. is maintained with oxygen. During 15 minutes, the remaining nitrous-N¹⁵₂ oxide is oxidized. The trap is then cooled with liquid air, and the excess oxygen is pumped off.

The nitric-N¹⁵ oxide is transferred to the small side-arm on a large flask (*in vacuo* with liquid air), and oxygen, equal to 60% of the volume of the initial nitrous-N¹⁵₂ oxide, is added. The flask is removed from the vacuum line, and some water is added through the stopcock. With gentle movement of the flask, the nitric-N¹⁵ oxide is taken up quickly, and after 24 hours its oxidation to nitric-N¹⁵ acid is complete. The acid is converted to the easily stored potassium salt. For example, 750 ml. (at 680 mm. and 20°) of nitrous-N¹⁵ oxide is oxidized to nitrogen-N¹⁵ dioxide in 4 hours, which gives 5.35 g. (95%) of potassium nitrate-N¹⁵.

(b) *Sodium Nitrite-N¹⁵*. In a large vacuum flask (1.2 l.), equipped with a side-arm containing 21 ml. of degassed sulfuric acid, is placed 4.5 g. of potassium nitrate-N¹⁵ and 80 g. of mercury (Note 2). After evacuation of the flask, the stopcock is closed, the flask is removed from the vacuum line, and with tipping and shaking of the flask, the sulfuric acid is slowly run in. After 2 hours, the contents of the flask are colorless and cold. The flask is again attached to the vacuum line, the line and a second flask (1 l.) are evacuated (Note 2), the nitric-N¹⁵ oxide is collected in the U-tube connecting the two flasks (*in vacuo* with liquid nitrogen), and nitrogen formed in the reduction is pumped off. With the aid of a small side tube, the nitric-N¹⁵ oxide is transferred to the second flask with liquid nitrogen, and the vacuum line is filled with pure oxygen. Oxygen, equal to one-fourth of the nitric-N¹⁵ oxide pressure previously read, is introduced into the flask, and the nitric-N¹⁵ oxide is allowed to vaporize. In the flask, is formed a stoichiometric equilibrium mixture:



The flask is removed from the vacuum system, and a solution of 1.96 g. of sodium hydroxide (96%) in 6 ml. of water is introduced through the

stopcock (Note 4). The sodium nitrite- N^{15} solution is washed into a small volumetric flask, and analyzed aliquots indicate that the solution contains 37.8 mmoles (85%) of sodium nitrite- N^{15} and 4.9 mmoles (11.5%) of sodium nitrate- N^{15} .

(c) *1-Phenylhydrazine-2- N^{15}* . With stirring, 3 g. of pure aniline is added to 30 ml. of concentrated hydrochloric acid which is cooled to -4° . The required amount of the sodium nitrite- N^{15} solution is added during 1 hour, with the temperature not above 3° . The clear, cold diazonium solution is added, with rapid stirring, to a cold suspension of 20 g. of stannous chloride dihydrate in 20 ml. of concentrated hydrochloric acid. After 2 hours in an ice-water bath, the 1-phenylhydrazine-2- N^{15} hydrochloride is collected and washed with a little cold hydrochloric acid.

The salt is dissolved in 60 ml. of 10% sodium hydroxide solution. The free 1-phenylhydrazine-2- N^{15} is extracted into a total of 80 ml. of ether, dried over potassium hydroxide and distilled. The yield is 1.95 g. (53%) of pure 1-phenylhydrazine-2- N^{15} (18.1 mmoles), b.p. 120° (14 mm.).

B. Notes

1. The nitrous- N_2^{15} oxide used in this work was obtained as a by-product in the preparation of N_2^{15} gas by the hypobromite oxidation of ammonium- N^{15} chloride.¹

2. A diagram of the apparatus is given in the original literature.

3. The pressure of the nitric oxide, in the reduction flask, is measured on the manometer for future use.

4. Absorption of the gas is accompanied by slight warming.

C. Other Preparations

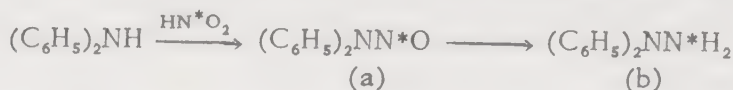
Potassium nitrate- N^{15} has been prepared by Bothner-By and Friedman² who used a glass electrode to titrate 1.67 *N* nitric- N^{15} acid to pH 7 with 2 *N* potassium hydroxide. They also prepared potassium nitrite- N^{15} from the nitrate- N^{15} using lead rather than mercury in the reduction. Although their yield (80.2%) was slightly less than that of Clusius and Hoch, their method is somewhat simpler. Vaughan, *et al.*,³ obtained a 64% yield of potassium nitrite- N^{15} using lead in a similar procedure.

¹K. Clusius, *Helv. Chim. Acta*, 33, 2144 (1950).

²A. A. Bothner-By and L. Friedman, *J. Am. Chem. Soc.*, 73, 5391 (1951).

³W. R. Vaughan, D. I. McCane and G. B. Sloan, *ibid.*, 73, 2298 (1951).

(1951).

1,1-DIPHENYLHYDRAZINE-2-N¹⁵ HYDROCHLORIDE

K. Clusius and M. Vecchi, *Helv. Chim. Acta*, 36, 933 (1953).

A. Procedure

(a) *N-Nitroso-N¹⁵-diphenylamine*. To a solution of 4.2 g. of diphenylamine in 30 ml. of ethanol is added 3 ml. of concentrated hydrochloric acid. To the resulting suspension of amine salt, cooled to -5° and stirred magnetically, is added dropwise a solution of 1.7 g. of sodium nitrite-N¹⁵ in 7 ml. of water. The salt dissolves gradually, and the nitrosamine later begins to separate. It is precipitated completely by the addition of water after the diazotization is finished. The yellow, crystalline product is recrystallized from alcohol; yield, 4.7 g. (95%).

(b) *1,1-Diphenylhydrazine-2-N¹⁵ Hydrochloride*. A solution of 4.7 g. of *N-nitroso-N¹⁵-diphenylamine* in 50 ml. of alcohol is mixed with 20 g. of zinc dust and, with vigorous stirring, 25 ml. of acetic acid is added dropwise during 45 minutes. The temperature of the mixture is not allowed to rise above 10° during this time. Then, the mixture is warmed very slowly to 30° (Note 1) and finally is heated to boiling. The solution is filtered, and the residue is extracted seven times with 20-ml. portions of hot alcohol.

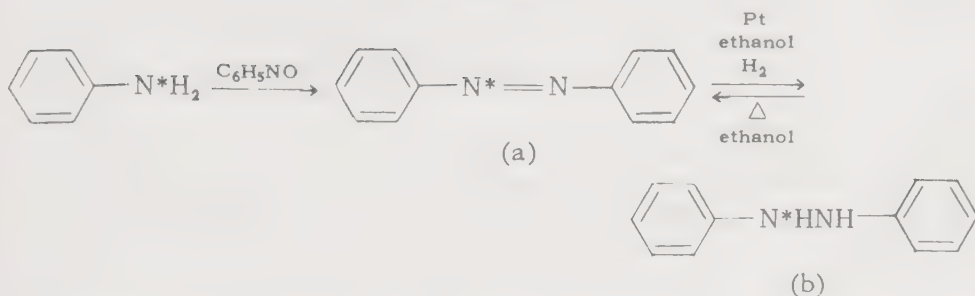
The combined filtrate is concentrated to one-third volume and mixed with an equal volume of water and 200 ml. of concentrated hydrochloric acid. After the mixture is kept at -10° for 5 hours, the salt is collected, dissolved in a minimum of alcohol and precipitated with ether at 0° . This procedure is repeated twice more, and the crystalline product is washed with dry ether. The yield is 4.0 g. (70%) (Note 2).

B. Notes

1. About 1 hour is necessary or the yield is poor.
2. The free base, 1,1-diphenylhydrazine-2-N¹⁵, was prepared in ether solution. A mixture of 0.55 g. of the hydrochloride salt and 20 ml. of hot 2 *N* sodium hydroxide was shaken for a few minutes in a small separatory funnel. The slightly yellow free base was then taken up in 50 ml. of ether, washed several times with water and dried over sodium sulfate.

HYDRAZOBENZENE- N_1^{15}

METHOD I



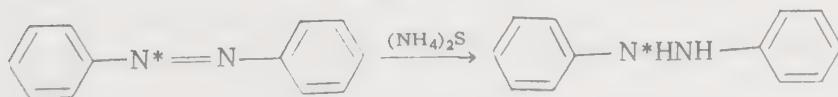
P. F. Holt and B. P. Hughes, J. Chem. Soc., 1953, 1666.

A. Procedure

(a) *Azobenzene- N_1^{15}* . A solution of 500 mg. of aniline- N^{15} and 620 mg. of nitrosobenzene in 10 ml. of methanol, containing 1 ml. of glacial acetic acid, is heated under reflux.¹ Steam distillation of the reaction mixture first removes unchanged nitrosobenzene; then, azobenzene- N_1^{15} distills more slowly. The latter fraction of distillate is extracted with petroleum ether to obtain azobenzene- N_1^{15} in 80% yield.

(b) *Hydrazobenzene- N_1^{15}* . A solution of 200 mg. of azobenzene- N_1^{15} in 5 ml. of dry ethanol is hydrogenated at normal pressure, in the presence of 5 mg. of platinum catalyst, until the color of azobenzene disappears. The solution is cooled in ice, centrifuged to remove catalyst and then immediately transferred to a Pyrex tube, which is cooled in Dry Ice, evacuated and sealed (Notes 1 and 2).

METHOD II



A. Procedure

A solution of 300 mg. of azobenzene- N_1^{15} in 15 ml. of ethanol is heated under reflux, and 15 ml. of ammonium sulfide solution is added through the condenser. After the solution is heated for 2 minutes, the flask is stoppered, and the mixture is cooled in a refrigerator for 1 hour. The precipitate of hydrazobenzene- N_1^{15} is collected and washed, first with distilled water and then with distilled water containing dissolved sulfur dioxide. The yield of product, m.p. $126-127^\circ$ after recrystallization from 50% aqueous ethanol, is 85-90%.

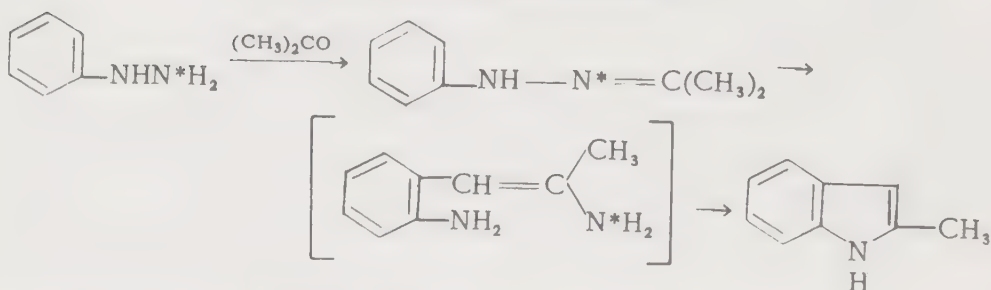
B. Notes

1. The solid hydrazobenzene- N_1^{15} was not isolated, but the yield was estimated to be 90% from the final yield of azobenzene- N_1^{15} derived from it. Hydrazobenzene- N_1^{15} was also prepared by reducing azobenzene- N_1^{15} in ethanol with zinc and sodium hydroxide. This product required purification; yield 70–75%.

2. The disproportionation of hydrazobenzene- N_1^{15} (200 mg.) was carried out in 5 ml. of ethanol heated at $150 \pm 1^\circ$ for 48 hours, in a sealed tube. The solution was mixed with dilute sulfuric acid in excess and steam-distilled to obtain azobenzene- N_1^{15} in 75% yield.

¹C. Mills, J. Chem. Soc., 1895, 925.

1-ISOPROPYLIDENE-2-PHENYLHYDRAZINE-1- N^{15}
(Acetone Phenylhydrazone-2- N^{15})



K. Clusius and H. R. Weissner, *Helv. Chim. Acta*, 35, 400 (1952).

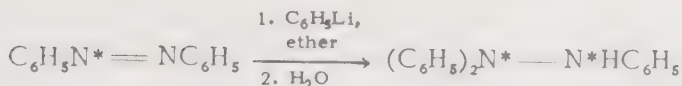
A. Procedure

1-Phenylhydrazine-2- N^{15} , 0.3 g., is mixed with 0.3 g. of acetone and heated on a boiling water-bath for 45 minutes (Note 1). The mixture is treated with 1 g. of anhydrous zinc chloride and heated in an oil-bath at 180° for one minute. After cooling the mixture, it is washed into a round-bottomed flask with 10 ml. of dilute hydrochloric acid, and the 2-methylindole is distilled with steam. The oily product, which soon solidifies, is crystallized from a little petroleum ether, m.p. 59° (Note 2).

B. Notes

1. Since this was a mechanism study the hydrazone was not isolated but very easily could have been.

2. The fact that the 2-methylindole did not contain excess N^{15} indicates that 2-(2-methyl-2-aminovinyl- N^{15})aniline is a plausible intermediate in the mechanism. Therefore, 1-phenylhydrazine-1- N^{15} would give 2-methylindole-1- N^{15} in the Fischer indole synthesis.

TRIPHENYLHYDRAZINE- $N_{1/2}^{15}$ 

P. F. Holt and B. P. Hughes, J. Chem. Soc., 1955, 1320.

A. Procedure

According to the method of Holt and Hughes,¹ 500 mg. of azobenzene- N_1^{15} is dissolved in 20 ml. of dry ether under nitrogen. To this solution cooled to 0° is added 230 mg. of phenyllithium in 5 ml. of dry ether. The mixture is stirred for 5 minutes, then saturated ammonium chloride solution is added. The ether layer is separated and evaporated to dryness. The residue is washed with petroleum ether and recrystallized from ethanol. The yield of triphenylhydrazine- $N_{1/2}^{15}$, m.p. $141-142^\circ$ (dec.), is 100 mg. (Note 1).

B. Notes

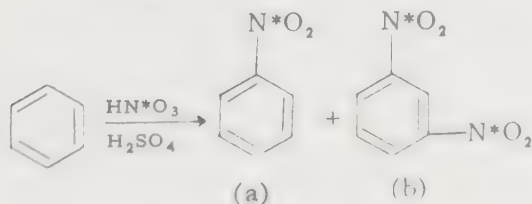
1. This product was used in a study of the thermal and photochemical decomposition of triphenylhydrazine. Thermal decomposition of triphenylhydrazine- $N_{1/2}^{15}$ gave azobenzene- N_2^{15} which was shown by analysis to have a random distribution of the isotopic nitrogen and therefore was formed from fragments containing only one nitrogen atom. This result differs from that obtained in the thermal or photochemical decomposition of hydrazobenzene- N_1^{15} which gives azobenzene- N_1^{15} since random pairing does not occur (see hydrazobenzene- N_1^{15} , Note 2). Other products formed in the thermal decomposition of triphenylhydrazine- $N_{1/2}^{15}$ are diphenylamine- N^{15} and a small amount of aniline- N^{15} .

The mechanism of the decomposition and the work of Wieland and Reverdy² and Goldschmidt³ are discussed by Holt and Hughes.

¹P. F. Holt and B. P. Hughes, J. Chem. Soc., 1954, 764.

²H. Wieland and A. Reverdy, Ber., 48, 1112 (1915).

³S. Goldschmidt, *ibid.*, 53, 59 (1920).

1,3-DINITROBENZENE- N_2^{15} 

K. Clusius and H. Craubner, Helv. Chim. Acta, 38, 1060 (1955).

A. Procedure (Note 1)

(a) *Nitrobenzene-N¹⁵*. To 12.5 ml. of concentrated sulfuric acid, 14 g. of nitric-N¹⁵ acid (prepared in a vacuum apparatus from 20.9 g. of potassium nitrate-N¹⁵ and 11.5 ml. of concentrated sulfuric acid) is added gradually, with shaking. After the mixture is cooled to room temperature, 10 ml. of benzene is added with stirring and cooling, if necessary, to maintain the temperature below 60°. The mixture is then heated at 50–60° for one-half hour, with stirring, in a flask equipped with a condenser. In a separatory funnel, the lower layer, which consists of sulfuric and nitric acids, is separated from the organic layer which contains nitrobenzene-N¹⁵ and *m*-dinitrobenzene-N₂¹⁵. The latter is washed with water, dilute sodium hydroxide and again with water (Note 2).

Fractionation of the mixture gives 7 g. of nitrobenzene-N¹⁵, (a), and 4.7 g. of *m*-dinitrobenzene-N₂¹⁵, (b), (Note 3).

B. Notes

1. Since very little experimental detail was given by Clusius and Craubner, the following procedure is according to Gattermann and Wieland.¹

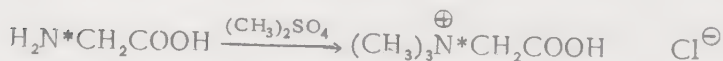
2. Nitrobenzene forms the lower layer with water.

3. Probably the fractionation would best be made under vacuum; the b.p. of nitrobenzene is 210–211°, while that of *m*-dinitrobenzene, which melts at 89.57°, is 302.8° (770 mm.).

¹L. Gattermann and H. Wieland, *Laboratory Methods of Organic Chemistry*, Macmillan and Co., London, 1941, p. 161.

BETAINE-N¹⁵ CHLORIDE

(Carboxymethyltrimethylammonium-N¹⁵ Chloride)



D. Stetten, Jr., *J. Biol. Chem.*, 138, 437 (1941).

A. Procedure

Betaine-N¹⁵ chloride is prepared from glycine-N¹⁵ according to an adaptation of the procedure of Novak.¹ In the alkylation of amino-monocarboxylic acids, 1 mole of the acid is reacted with 4.5 moles of alkyl sulfate and 4.5 moles of potassium hydroxide. Glycine is dissolved in the minimum amount of water and neutralized with potassium hydroxide. Then, at room temperature, the concentrated potassium hydroxide and methyl sulfate are added in small portions, such that the solution is always alkaline. With each addition, the mixture is shaken until the methyl sulfate disappears (Note 1). Finally, the alkaline solution is heated for

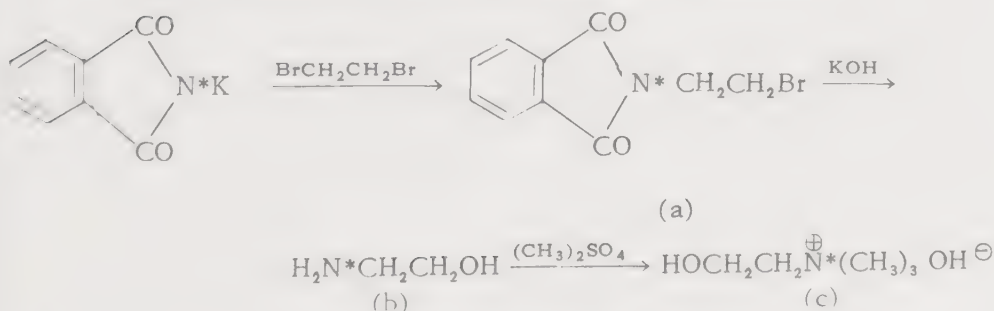
20 minutes (Note 2), neutralized with dilute sulfuric acid and evaporated to a syrup on a water-bath. The residue is twice extracted with cold 95% ethanol (Note 3). The extracts are combined, diluted with an equal volume of water and again evaporated on the water-bath to a syrup. This residue is once more extracted with 95% ethanol which upon evaporation leaves a heavy, yellow syrup that is quite soluble in absolute alcohol. The syrup is heated with dilute hydrochloric acid to convert potassium methyl sulfate to the free acid, which is precipitated by the addition of barium chloride. The filtrate is concentrated and heated several hours, and again treated with barium chloride. The filtrate is evaporated to a thick syrup on the water-bath and extracted with 95% alcohol to eliminate the excess barium chloride. The alcoholic solution of the alkylated amino acid hydrochloride is evaporated to dryness. The residue is extracted with absolute ethanol (Note 4). The majority of the material, insoluble in absolute alcohol, is recrystallized from water in large plates, m.p. 228.4°. The yield of betaine chloride is 93–94%.

B. Notes

1. When methyl sulfate is the alkylating agent, it may be necessary to cool the flask with tap water.
2. This ensures complete destruction of any unreacted methyl sulfate.
3. Potassium methyl sulfate and potassium sulfate are only slightly soluble in 95% ethanol.
4. The soluble portion is carbomethoxymethyltrimethylammonium chloride; yield 1.3%.

¹J. Novak, Ber., 45, 834 (1912).

CHOLINE-N¹⁵ (2-Hydroxyethyltrimethylammonium-N¹⁵ Hydroxide)



K. Bloch and R. Schoenheimer, J. Biol. Chem., 138, 186 (1941).

Procedure

(a) N-(2-Bromoethyl)phthalimide-N¹⁵. The isotopic bromoethylphthalimide is prepared as described by Salzberg¹ from 37 g. of potassium

phthalimide- N^{15} and 90 g. of ethylene bromide. The yield is 33.8 g. (67%).

(b) *2-Aminoethanol- N^{15}* . The *N*-(2-bromoethyl)phthalimide- N^{15} , 33.8 g., is hydrolyzed with 30% potassium hydroxide solution according to Putokhin.² The 2-aminoethanol- N^{15} is distilled from the reaction mixture into an excess of dilute hydrochloric acid. 2-Aminoethanol- N^{15} hydrochloride is isolated from alcoholic solution by precipitation with ether. The yield is 9.23 g. (71%).

(c) *Choline- N^{15}* , (*2-Hydroxyethyltrimethylammonium- N^{15} Hydroxide*). To a cooled solution of 3.9 g. of 2-aminoethanol- N^{15} hydrochloride in 170 ml. of 5% potassium hydroxide solution, 25.5 g. of methyl sulfate is added gradually with stirring. The stirring is continued for 1 hour, and the filtered solution is then made acid to Congo red indicator with hydrochloric acid. On the addition of 15 g. of gold chloride, 14.2 g. (80%) of choline- N^{15} gold chloride is obtained, m.p. 268° with decomposition.

¹*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1932, p. 114.

²N. Putokhin, *Trans. Inst. Pure Chem. Reagents*, 6, 10 (1927); through *Chem. Abstracts*, 23, 2938 (1927).

CHOLINE- N^{15} CHLORIDE

(2-Hydroxyethyltrimethylammonium- N^{15} Chloride)



J. A. Muntz, *J. Biol. Chem.*, 182, 491 (1950).

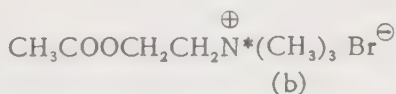
Procedure

Trimethylamine- N^{15} is distilled into a glass bomb-tube cooled to -30° and containing ethylene chlorohydrin. The tube is sealed and heated for 3 hours in a boiling water-bath. The tube is opened, and the contents are dissolved in absolute ethanol. The choline- N^{15} chloride is precipitated by diluting the alcoholic solution with 10 volumes of absolute ether. It is purified by repeated dissolution in absolute alcohol and precipitation with ether.

ACETYLCHOLINE-N¹⁵ BROMIDE
(2-Acetoxyethyltrimethylammonium-N¹⁵ Bromide)



(a)



(b)

M. A. Rothenberg, D. B. Sprinson and D. Nachmansohn, *J. Neurophysiol.*, **11**, 112 (1948).

A. Procedure

(a) *Trimethylammonium-N¹⁵ Chloride*. This amine is prepared by adaptation of the method of Sommelet and Ferrand¹ with slight modification according to Clark.² Ammonium-N¹⁵ chloride, 5.4 g. (0.1 mole), is dissolved with cooling in 25.5 g. of 90% formic acid solution (0.5 mole). Following the addition of 22.5 g. of 40% formaldehyde solution (0.3 mole), the mixture is heated under reflux on the steam-bath for 10 hours. Slightly more than 0.1 mole of hydrochloric acid is added, and the mixture is evaporated to dryness. The trimethylamine-N¹⁵ is then liberated from the residue by the dropwise addition of 40% sodium hydroxide and collected in 0.1 mole of hydrochloric acid. The solution is evaporated on a steam-bath, and the trimethylammonium-N¹⁵ chloride is dried at 110°. The yield is 7.2 g. (75.4%), m.p. 271-275°.

(b) *Acetylcholine-N¹⁵ Bromide, (2-Acetoxyethyltrimethylammonium-N¹⁵ Bromide)*. The trimethylamine-N¹⁵ is liberated with alkali from 0.675 g. (0.007 mole) of the hydrochloride and distilled into an ether solution of 1.25 g. (0.0075 mole) of 2-bromoethyl acetate, which is cooled with a Dry Ice-acetone bath. After a few hours, the acetylcholine-N¹⁵ bromide is collected on a filter and recrystallized from alcohol. After cooling the solution, the white crystalline material is quickly collected on a filter using vacuum and is dried in a vacuum desiccator over phosphorus pentoxide.

B. Other Preparations

Trimethylamine-N¹⁵ has been prepared³ from the reaction of ammonium-N¹⁵ chloride with paraformaldehyde according to the procedure of Adams and Marvel.⁴

¹M. Sommelet and M. Ferrand, *Bull. soc. chim. France*, **35**, 446 (1924).

²H. T. Clark, H. B. Gillespie and S. Z. Weiss Haus, *J. Am. Chem. Soc.*, **55**, 4571 (1933).

³J. A. Muntz, *J. Biol. Chem.*, **182**, 491 (1950).

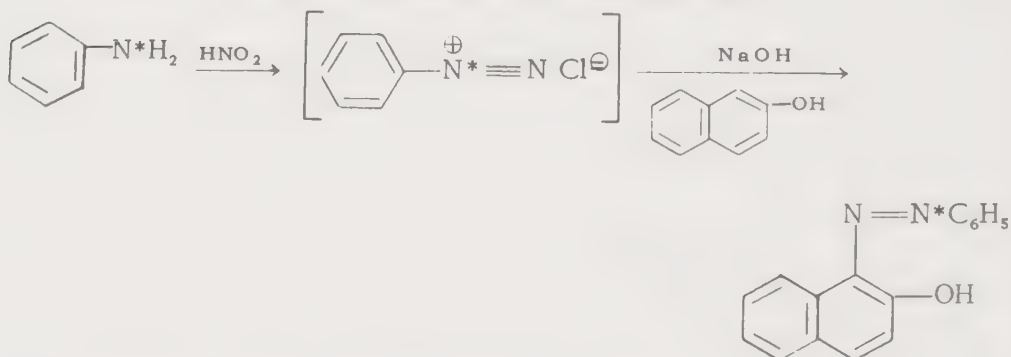
⁴*Organic Syntheses*, Coll. Vol. I, Wiley, New York, 1932, p. 517.

TETRAMETHYLAMMONIUM NITRATE- N^{15} 

K. Clusius and M. Vecchi, *Helv. Chim. Acta*, 36, 930 (1953).

Procedure

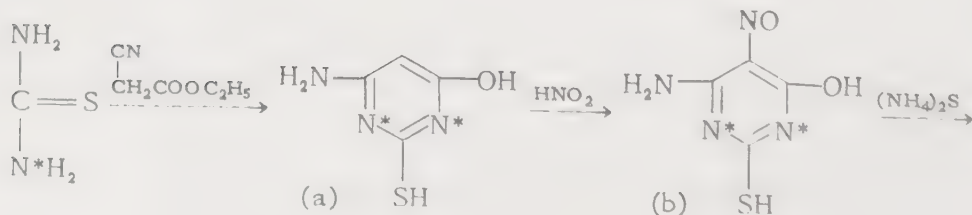
To a solution of tetramethylammonium iodide is added an equivalent amount of silver hydroxide. After the removal of silver iodide, the filtrate is acidified with dilute nitric- N^{15} acid and evaporated to dryness. The residue is redissolved in dilute nitric- N^{15} acid and again taken to dryness. The resulting tetramethylammonium nitrate- N^{15} is free of silver and iodide ions.

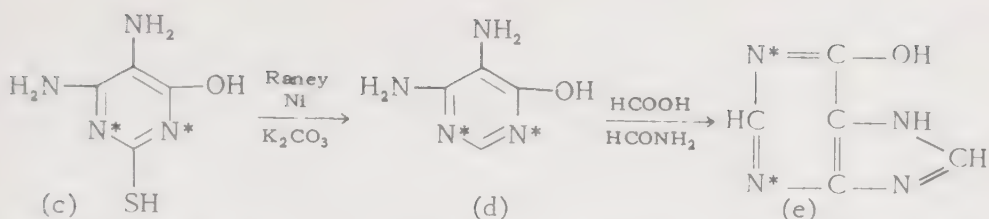
1-(PHENYLazo-2- N^{15})-2-NAPHTHOL

P. F. Holt and B. I. Bullock, *J. Chem. Soc.*, 1950, 2310.

Procedure

To a solution of 0.46 g. of aniline- N^{15} in 2 ml. of dilute hydrochloric acid (1:1), 40% sodium nitrite solution is added dropwise until free nitrous acid is detected. The solution is added to 0.6 g. of 2-naphthol in 3 ml. of 10% sodium hydroxide; the precipitate of the azo compound is collected, washed with water and dried *in vacuo*. The yield of crude product is 0.83 g.

HYPOXANTHINE-1,3- $N^{15}_{1/2}$ 



H. Getler, P. M. Roll, J. F. Tinker and G. B. Brown, J. Biol. Chem., 178, 262 (1949).

A. Procedure

(a) *6-Amino-2-mercapto-4-pyrimidinol-1,3-N₁₅*. According to the procedure of Traube,¹ 4.6 g. of sodium is dissolved in 100–200 ml. of absolute alcohol, and to this solution, are added 16 g. of finely powdered dry thiourea and 22 g. of ethyl cyanoacetate. This mixture is heated under reflux on a water-bath for 2 hours. The ethyl sodiumcyanoacetate soon goes into solution, and after a short period of heating the sodium salt of the hydroxypyrimidine begins to crystallize. The crystals are removed from the mother liquor, which is evaporated to dryness. Then, the crystals together with the residue are dissolved in water, and the solution is made distinctly acidic with dilute acetic acid. The aminomercaptopyrimidinol separates in fine, colorless needles; the yield is 25–27 g. The crude material is recrystallized from hot water in needles containing 1 molecule of water of crystallization.

(b) *6-Amino-2-mercapto-5-nitroso-4-pyrimidinol-1,3-N₁₅*. According to Traube,¹ 20 g. of the above compound is dissolved in 0.5 l. of water which contains 5.5 g. of sodium hydroxide. To this solution is added 10 g. of sodium nitrite and then 18 g. of acetic acid. The pyrimidinol first crystallizes in fine needles and then reacts slowly with nitrous acid. After a few hours, the nitroso compound is brought into solution by the addition of sodium hydroxide. The ammonium salt is then precipitated by the addition of ammonium chloride. The product is collected, washed with water, alcohol and ether and dried; the yield is 21 g.

(c) *5,6-Diamino-2-mercapto-4-pyrimidinol-1,3-N₁₅*. The ammonium salt of the nitroso compound, 20 g., is added to boiling 5% ammonium sulfide solution in small portions.¹ The addition of the ammonium salt is continued as long as hydrogen sulfide is evolved and then fresh ammonium sulfide is added as needed. At the end of the reduction there should not be an excessive amount of hydrogen sulfide remaining in the solution. To obtain the free base, the solution is filtered to remove sulfur and evaporated. As the solution loses ammonia, the product, which is slightly soluble in water and in alcohol, crystallizes.

(d) *5,6-Diamino-4-pyrimidinol-1,3-N₁₅ Hydrochloride*. According to a modification of the procedure of Robbin,² a mixture of 1.85 g. of 5,6-diamino-2-mercapto-4-pyrimidinol-1,3-N₁₅, 1.0 g. of anhydrous potassium carbonate, 4 g. of Raney nickel, and 10 ml. of water is heated under re-

flux for 2 hours. Carbon is added, and the solution is warmed and filtered. The filtrate is cooled, saturated with hydrogen chloride and again cooled, and the product is collected. The product, 1.43 g., contains some potassium chloride. A second crop of crude 5,6-diamino-4-pyrimidinol-1,3- $N_{1/2}^{15}$ hydrochloride, 0.64 g., is obtained by the addition of alcohol to the filtrate.

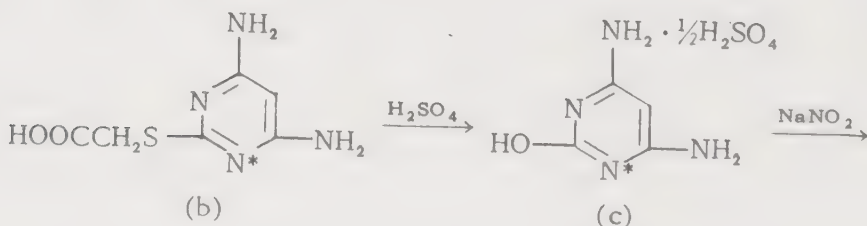
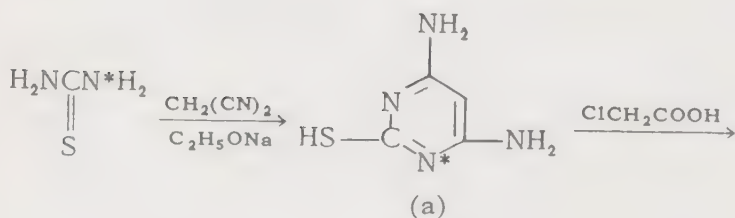
(e) *Hypoxanthine-1,3- $N_{1/2}^{15}$* , [6(1H)-Purinone-1,3- $N_{1/2}^{15}$]. Formation of the imidazole ring³ is accomplished by heating 1.43 g. of crude 5,6-diamino-4-pyrimidinol-1,3- $N_{1/2}^{15}$ hydrochloride, 11 ml. of formic acid and anhydrous formamide in a sealed tube at 160° for 2 hours. The contents of the tube are evaporated to dryness on a steam-bath under a stream of air. The residue is recrystallized from 60 ml. of water after treatment with carbon. The yield of hypoxanthine-1,3- $N_{1/2}^{15}$ is 0.31 g.

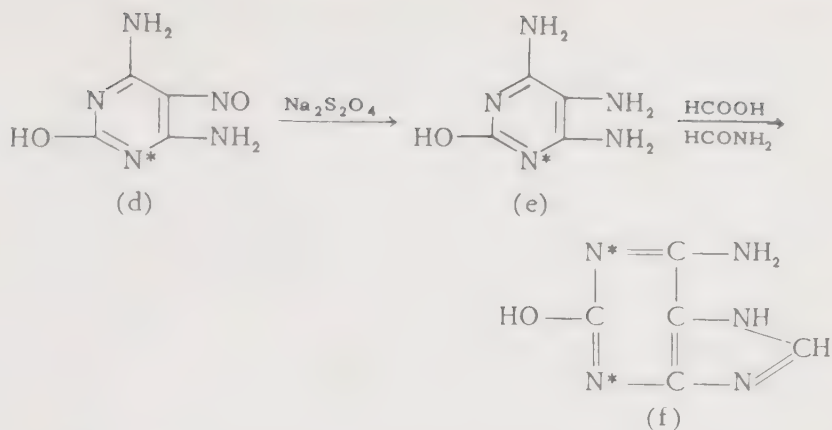
¹W. Traube, *Ann.*, 331, 73 (1904).

²R. O. Robbin, Jr., J. O. Lampen, J. P. English, Q. P. Cole and J. R. Vaughan, Jr., *J. Am. Chem. Soc.*, 67, 292 (1945).

³A. Bendich, J. F. Tinker and G. B. Brown, *ibid.* 70, 3109 (1948).

ISOGUANINE-1,3- $N_{1/2}^{15}$





A. Bendich, J. F. Tinker and G. B. Brown, J. Am. Chem. Soc., 70, 3110 (1948).

A. Procedure

(a) *4,6-Diamino-2-pyrimidinethiol-1-N¹⁵*. In the following modification of Traube's¹ synthesis, 50.0 g. (0.76 mole) of malononitrile is added to 60.0 g. (0.79 mole) of thiourea-N¹⁵ in 400 ml. of absolute ethanol containing 18.0 g. (0.783 mole) of sodium (Note 1). The mixture is refluxed for 2 hours. Complete solution is effected by adding three volumes of water, and the solution is neutralized with glacial acetic acid to obtain 74.0 g. (70%) of white needles. This material is recrystallized from water.

(b) [*4,6-Diamino-2-(pyrimidinylthio-1-N¹⁵)*]acetic Acid (Note 2). To 10 g. of 4,6-diamino-2-pyrimidinethiol-1-N¹⁵ in 140 ml. of cold water is added 10 g. of chloroacetic acid. The mixture is heated under reflux, with constant stirring, for 1 hour. A copious white precipitate soon begins to form. The product, collected after chilling the mixture, weighs 11.0 g. (79%). Colorless needles are obtained upon recrystallization from water. Recrystallization from 2 N sulfuric acid results in hexagonal prisms of the sulfate salt, which are dried at room temperature over phosphorus pentoxide *in vacuo* (Note 3).

(c) *4,6-Diamino-2-pyrimidinol-1-N¹⁵ Sulfate*. To 5.4 g. of 4,6-diamino-2-pyrimidinethiol-1-N¹⁵ and 5.5 g. of chloroacetic acid is added 75 ml. of boiling water, and the solution is heated under reflux for 1½ hours (Note 4). Without cooling, 9.5 ml. of 18 N sulfuric acid is added, and heating is continued for 1 hour (Note 5). Decolorizing carbon is added, the solution is filtered, and upon cooling the filtrate, 5.02 g. (77%) of white elongated prisms are deposited (Note 6).

(d) *4,6-Diamino-5-nitroso-2-pyrimidinol-1-N¹⁵*. 4,6-Diamino-2-pyrimidinol-1-N¹⁵ sulfate, 5.0 g. (0.029 mole), dissolved in 670 ml. of boiling water, is treated with 3.0 g. of sodium nitrite dissolved in 50 ml. of water. After 5 minutes, an equal volume of crushed ice is added, and the deposit of

red nitroso compound is collected, washed with cold water and dried; yield 3.8 g. (86%).

(e) 4,5,6-Triamino-2-pyrimidinol-1- N^{15} . The nitroso compound, 3.8 g., is suspended in 180 ml. of water, 5.5 g. of sodium hydrosulfite is added, and the mixture is boiled for 3 minutes. Then, 23 ml. of 18 *N* sulfuric acid is cautiously added and the solution is treated with carbon and quickly filtered. Upon cooling, 3.91 g. (67%) of white needles is formed. The sulfate may be formed by recrystallization of this material from 2 *N* sulfuric acid. It is dried at 130° *in vacuo* over phosphorus pentoxide.

(f) Isoguanine-1,3- $N^{15}_{1/2}$, (6-Amino-2-purinol-1,3- $N^{15}_{1/2}$). 4,5,6-Triamino-2-pyrimidinol-1- N^{15} sulfate, 2.84 g. (0.012 mole), 35 ml. of formamide and 0.85 ml. of 98% formic acid (0.022 mole) are heated in a bomb tube at 160° for 3 hours. Upon cooling the mixture, 1.67 g. (93%) of the crystalline free base is deposited (Note 7). White, prismatic needles of the sulfate monohydrate are obtained by recrystallization of the free base from 5% sulfuric acid (Note 8).

B. Notes

1. The experimental details given do not include the use of isotopic nitrogen. The authors state that isoguanine-1,3- $N^{15}_{1/2}$ was prepared according to this procedure.

2. When an attempt was made to convert 4,6-diamino-2-pyrimidinethiol to the corresponding diamino-2-hydroxy compound, by adapting the chloroacetic acid desulfurization method of Wheeler and Liddle,² the intermediate carboxymethyl thioether separated.

3. The molecular formula is $(C_6H_8O_2N_4S)_2 \cdot H_2SO_4 \cdot H_2O$.

4. Very little solid separates.

5. An odor reminiscent of thioglycolic acid is noted throughout this period.

6. The molecular formula is $(C_4H_6N_4O)_2 \cdot H_2SO_4$. The hydrochloride is obtained upon recrystallization from 2 *N* hydrochloric acid.

7. Ammonium sulfate precipitates from the filtrate upon addition of alcohol.

8. The sulfate salt does not lose its water of crystallization upon heating at 130° *in vacuo* for 3 hours.

C. Other Preparations

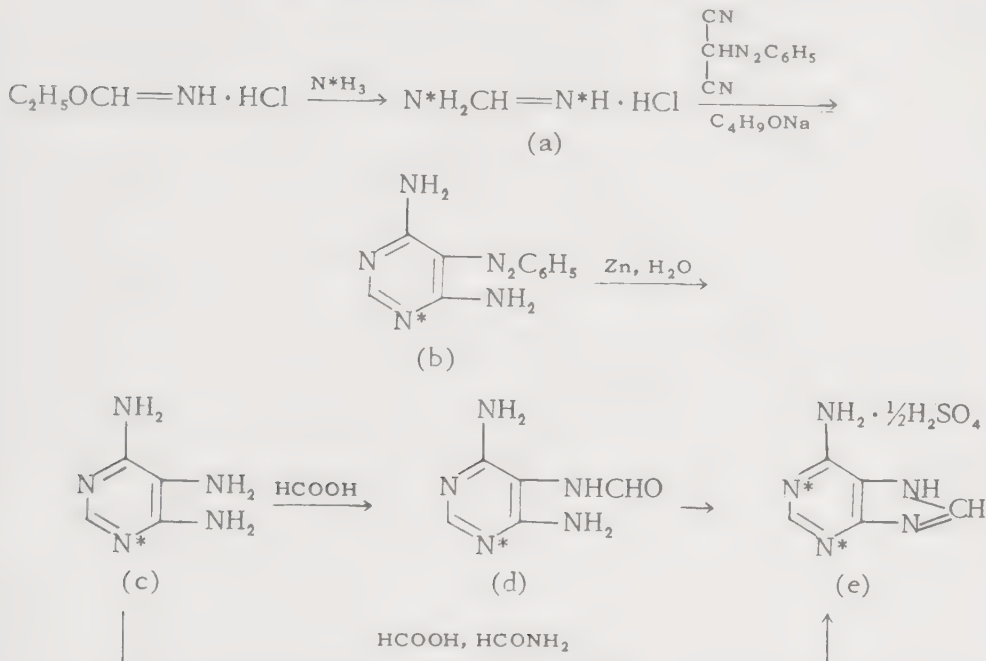
Bendich, *et al.*, obtained 4,6-diamino-2-pyrimidinol, in fair yield, by heating (4,6-diamino-2-pyrimidinylthio)acetic acid with 2 *N* sulfuric acid.

4,6-Diamino-5-nitroso-2-pyrimidinol is also formed by treating the 4,6-diamino compound with nitrous acid in 50% acetic acid solution.

¹W. Traube, *Ann.*, 331, 64 (1904).

²H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, 40, 547 (1908).

ADENINE-1,3-N¹⁵ SULFATE
(6-Aminopurine-1,3-N¹⁵ Sulfate)



L. F. Cavalieri, J. F. Tinker and A. Bendich, J. Am. Chem. Soc., 71, 533 (1949).

A. Procedure

(a) *Formamidine-N¹⁵_{1/2} Hydrochloride*. Ethyl formimidate hydrochloride, 17.0 g. (0.18 mole), and 35 ml. of absolute ethanol are placed in a dry Carius tube equipped with an inlet tube reaching an inch below the surface of the ethanol (Note 1). The Carius tube is immersed in a Dry Ice-bath and connected to an ammonia generator. The ammonia-N¹⁵ from 8.8 g. (0.11 mole) of ammonium-N¹⁵ nitrate is swept into the solution with a stream of air (Note 2), which then passes through an acid trap containing 5% aqueous boric acid and methyl violet indicator.

The bomb tube, with its inlet tube detached and included, is sealed and heated for two hours at 100°, during which time the tube is shaken occasionally. The tube is cooled and opened, and any excess ammonia is collected in the aqueous boric acid trap by drawing a stream of dry air through an inlet tube for thirty minutes while the tube is surrounded by a bath at 55–60° (Note 3). The hot ethanolic solution is filtered (Note 4), the alcohol is evaporated by means of a stream of dry air, and the residual formamidine-N¹⁵_{1/2} hydrochloride is dried *in vacuo* over phosphorus pentoxide.

(b) *4,6-Diamino-5-phenylazopyrimidine-1-N¹⁵*. In a 1-l. Erlenmeyer flask is placed 29 ml. (46.5 g., 0.15 mole) of aniline and 85 ml. (1.02

moles) of concentrated hydrochloric acid. Ice is added to the mixture to maintain a temperature of 0 to 5°. With stirring, 29.0 g. (0.50 mole, assay 97%) of sodium nitrite in 50 ml. of water is added in small portions. After ten minutes, 51 g. (0.63 mole) of anhydrous sodium acetate in 100 ml. of water is added, followed by 33 g. (0.50 mole) of malononitrile¹ in 25 ml. of ethanol. After 30 minutes, the phenylazomalononitrile is collected by filtration, washed with cold water and dried in air. The yield of material recrystallized from benzene (5 ml. per gram) is 48-52 g. (55-60%) (Note 5).

A three-necked flask is provided with a Hershberg stirrer, a reflux condenser (protected with a drying tube) and two separatory funnels, in series. Formamidine- $N_{1/2}^{15}$ hydrochloride prepared from 0.11 mole of ammonia- N^{15} is placed in the flask. To this is added 20 g. (0.118 mole) of phenylazomalononitrile in 30 ml. of 1-butanol (Note 6). In the bottom separatory funnel (equipped with a drying tube) is placed 2.9 g. (0.13 mole) of sodium. Dry 1-butanol (100-150 ml.) from the upper funnel is added to the lower funnel in small portions. The sodium butoxide solution is added to the reaction mixture in several portions, and the mixture is refluxed gently for 4 hours. The mixture is cooled to 5°, and the solid is collected on a filter. The product is washed alternately with water and alcohol and dried for a short time at 110°; yield, 15-18 g. (70-80%). This material is used in the next step without further purification (Note 7).

(c) *4,5,6-Triaminopyrimidine-1- N^{15} Sulfate*. 4,6-Diamino-5-phenylazopyrimidine-1- N^{15} , 4.0 g. (0.0186 mole), is added to a boiling solution of 40 ml. of water and 6.0 ml. of ethyl cellosolve containing 4 g. of zinc dust. The mixture is boiled for 60-90 seconds with stirring; then 6 ml. of 18 *N* sulfuric acid is added to the hot solution as quickly as possible, and the mixture is decolorized with carbon and filtered immediately (Note 8). Upon cooling the solution, 2.4-3.4 g. (55-78%) of product precipitates. The 4,5,6-triaminopyrimidine-1- N^{15} sulfate thus obtained is used in the next section. It can be recrystallized from 2 *N* sulfuric acid (20 ml. per gram) with an 85% recovery.

(d) *4,6-Diamino-5-formamidopyrimidine-1- N^{15}* . 4,5,6-Triaminopyrimidine-1- N^{15} , 1.0 g. (0.008 mole), is warmed gently with 2 ml. of 98% formic acid until all the solid has gone into solution. The excess formic acid is removed by evaporation at room temperature, and the residue is recrystallized from ethanol; yield, 1.15 g. (95%).

(e) *Adenine-1,3- $N_{1/2}^{15}$ Sulfate*, (*6-Aminopurine-1,3- $N_{1/2}^{15}$ Sulfate*). Although the 5-formylamino compound is converted to adenine in good yield (77-87%), the superior procedure is the one-step ring closure with formamide and formic acid. 4,5,6-Triaminopyrimidine-1- N^{15} sulfate, 0.78 g., and 9 ml. of anhydrous formamide containing 0.3 ml. of 98% formic acid are heated in a bomb tube at 160-165° for 2.5 hours. The contents

of the tube are chilled, and the insoluble material is collected on a filter. The filtrate is evaporated to dryness *in vacuo* at about 150°. The total solid material is recrystallized from about 12 ml. of 2 N sulfuric acid; yield, 0.62 g. (95%).

B. Notes

1. The inlet tube is arranged for easy detachment.
2. The flow rate must not be so rapid that ammonia escapes condensation in the bomb tube.
3. Usually with this ratio of reactants no excess ammonia-N¹⁵ is collected. Ammonia-N¹⁵ which is not recoverable varies from 0 to 8%.
4. The residue is 99% pure ammonium chloride.
5. The crude material can be used in the following reaction.
6. The butanol is dried by distillation. When carefully dried ethanol is used as solvent for the reaction, the yield is frequently as low as 30%.
7. The 4,6-diamino-5-phenylazopyrimidine is difficult to recrystallize.
8. A lower yield results when the material is left in contact with acid.

C. Other Preparations

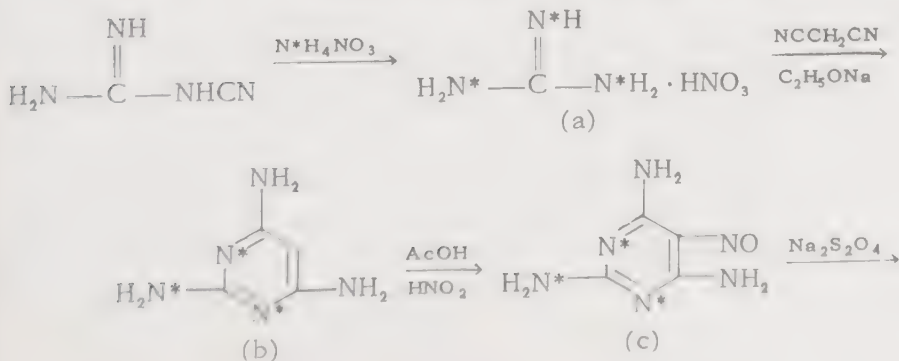
4,6-Diamino-5-phenylazopyrimidine-1-N¹⁵ has been catalytically hydrogenated² to give 4,5,6-triaminopyrimidine-1-N¹⁵ according to the procedure of Baddiley.³

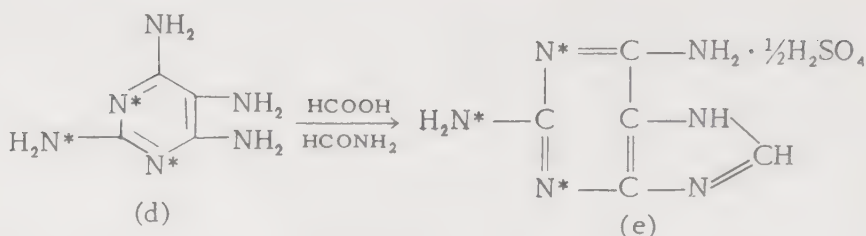
Adenine-1,3- $N_{1/2}^{15}$ has also been prepared¹ from 4,5,6-triaminopyrimidine-1- N^{15} via 4,6-diamino-5-thioformamidopyrimidine-1- N^{15} which is cyclized by heating under reflux with either water or pyridine.

¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 379.

²G. B. Brown, P. M. Roll, A. A. Plentl and L. F. Cavalieri, J. Biol. Chem., 172, 476 (1948).

³J. Baddiley, B. Lythgoe and A. R. Todd, J. Chem. Soc., 1943, 386.

2,6-DIAMINOPURINE-1,2,3-N₃¹⁵ SULFATE



A. Bendich, S. S. Furst and G. B. Brown, *J. Biol. Chem.*, **185**, 428 (1950).

A. Procedure

(a) *Guanidinium- N_3^{15} Nitrate*. This compound is prepared from cyanoguanidine and ammonium- N^{15} nitrate according to the following procedure of Davis¹ (Note 1). In a round-bottomed flask is placed an intimate mixture of 210 g. of cyanoguanidine (2.5 moles) and 440 g. of ammonium nitrate (5.5 moles) (Note 2). The flask is placed in an oil-bath at 110–120°, and the temperature of the oil is raised during 30 minutes to 160°. This bath temperature is maintained for 3 hours (Note 3). During the first hour the mass melts to a clear liquid which begins to deposit crystals and finally sets to a solid cake. At the end of three hours the flask is removed from the bath; the product is allowed to cool and is extracted on the steam-bath with successive quantities of water (about 2 l. is necessary to bring all the soluble material into solution) (Note 4). The solution is filtered to remove white amorphous insoluble material (ammeline and ammelide) (Note 5). The filtrate contains guanidinium nitrate along with small amounts of ammonium nitrate and biguanide nitrate. The solution is concentrated to 1 l., and the guanidinium nitrate, which crystallizes on cooling, is collected on a filter. A second crop is obtained by concentrating the filtrate to 250 ml. (Note 6). The combined yield of crude guanidinium nitrate is 520–560 g. (85–92%). The product is purified by recrystallization from 1 l. of water. The yield of purified product melting at 213–214° amounts to 500–520 g.

(b) *2,4,6-Triaminopyrimidine-1,2,3- N_3^{15}* . The following substituted pyrimidines are prepared according to a modification² of the method of Traube.³ To a solution of sodium ethoxide, prepared by dissolving 200 g. (8.7 moles) of sodium in 6 liters of absolute ethanol, 762 g. (6.25 moles) of guanidinium nitrate and 450 g. (6.8 moles) of malononitrile are added. The mixture is heated under reflux on a water-bath for 6 hours with stirring. The mixture is then cooled to room temperature, and the solid, which consists of sodium chloride and 2,4,6-triaminopyrimidine, is collected by filtration and washed with absolute ethanol.

(c) *2,4,6-Triamino-5-nitrosopyrimidine-1,2,3- N_3^{15}* . On this scale, one-half of the triaminopyrimidine is placed in a 4-l. beaker, and 1 l. of water is added. Vigorous mechanical stirring results in a homogeneous mix-

ture to which is added glacial acetic acid until the mixture is neutral to litmus. Further addition of 200 ml. of acetic acid results in complete solution. A solution of 220 g. (3.2 moles) of sodium nitrite in 500 ml. of water is added while vigorous stirring is continued. The bright red solid which has formed is filtered, as quickly as possible to avoid continued contact with excess nitrous acid, washed with water and pressed to drain off most of the water. The partially dried solid, 2,4,6-triamino-5-nitrosopyrimidine, is again divided into two parts for ease in handling.

(d) *2,4,5,6-Tetraminopyrimidine-1,2,3-N₃¹⁵ Sulfate*. Each portion of the nitroso compound is suspended in 1 l. of water which is heated to 60–70° with mechanical stirring. Solid sodium hydrosulfite is added slowly until the red color disappears (Note 7). The mixture is then heated to boiling and filtered through a steam-jacketed Büchner funnel. The filtrate, upon cooling in the refrigerator, deposits 755 g. (54%) of a yellow product. Repeated recrystallization from water gives orange needles, which progressively darken without melting.

(e) *2,6-Diaminopurine-1,2,3-N₃¹⁵ Sulfate*. In the procedure of Bendich,⁴ 1.33 g. of tetraminopyrimidine sulfate is heated at 160° for 3 hours in a sealed tube with 15 ml. of formamide and 0.4 ml. of 98% formic acid. Upon cooling, 1.53 g. of yellow crystals are deposited which are recrystallized from 5% sulfuric acid to yield white needles. The product is dried at 140° *in vacuo* over phosphorus pentoxide. The isotopic 2,6-diaminopurine is prepared in 88% yield from the tetraaminopyrimidine. It is recrystallized from 0.2 N sulfuric acid and dried over phosphorus pentoxide at room temperature in a vacuum desiccator. The molecular formula of the sulfate salt is $(C_5H_6N_6)_2 \cdot H_2SO_4 \cdot H_2O$.

B. Notes

1. This entire synthesis of 2,6-diaminopurine-1,2,3-N₃¹⁵ was carried out by Bendich, *et al.*; however, they have given literature references and no experimental details. Procedures taken from the original literature are given here.

2. A 10 per cent excess of ammonium nitrate is used because the biguanide mononitrate which is formed as an intermediate is strongly basic and tends to attack the unreacted ammonium nitrate, as indicated by the liberation of ammonia. The excess of ammonium nitrate is easily separated from the guanidinium nitrate by recrystallization from water.

3. When the molten mass reaches 160° its temperature begins to rise above that of the bath, generally reaching 200° during the course of 5 or 6 minutes. The mass should not be stirred or the temperature may run somewhat higher.

4. The hard cake goes into solution slowly, and time must be given for each portion to become saturated.

5. When the hot filtrate cools, it deposits flocks of ammelide along with crystals of guanidinium nitrate. Water may be added until the crystals dissolve, and the cold solution is again filtered to remove ammelide.

6. At this point, the mother liquor contains only ammonium nitrate, in significant amount, which may be recovered.

7. Each portion requires approximately 12.5 moles.

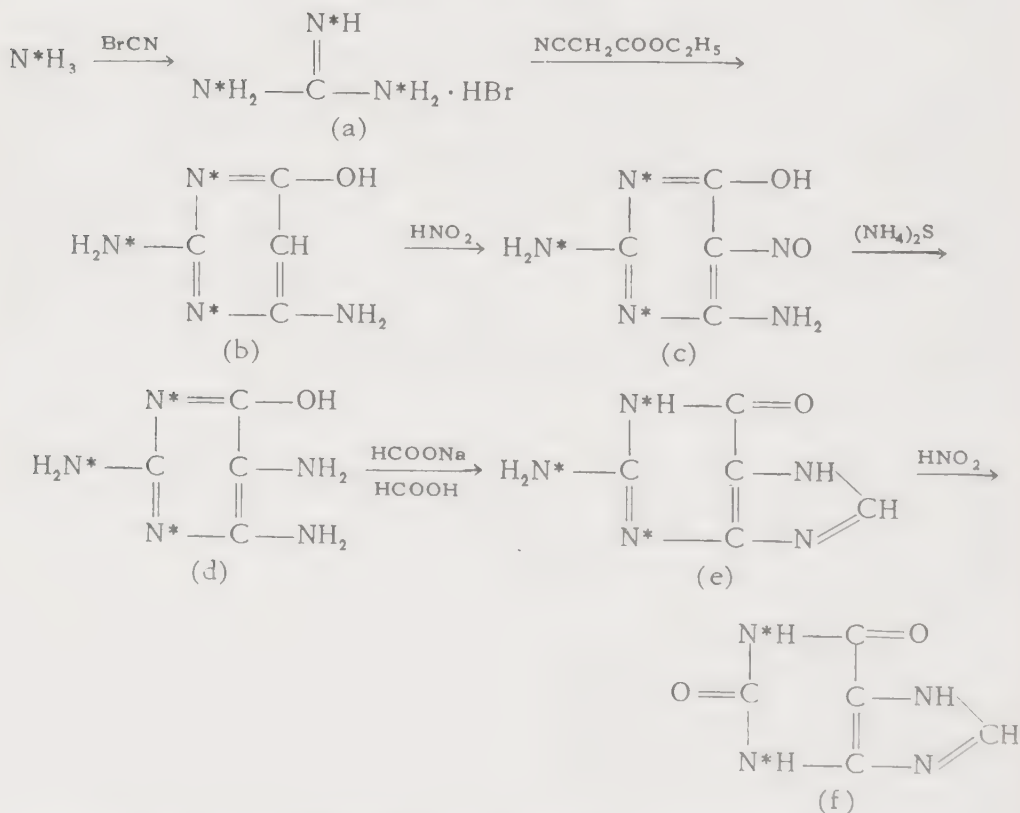
¹*Organic Syntheses*, Coll. Vol. I, 2nd ed., Wiley, New York, 1941, p. 302.

²M. F. Mallette, E. C. Taylor and C. K. Cain, *J. Am. Chem. Soc.*, 69, 1815 (1947).

³W. Traube, *Ber.*, 37, 4544 (1904).

⁴A. Bendich, J. F. Tinker and G. B. Brown, *J. Am. Chem. Soc.*, 70, 3113 (1948).

XANTHINE-1,3-N¹⁵
[2,6(1*H*,3*H*)-Purinedione-1,3-N₂¹⁵]



A. A. Plentl and R. Schoenheimer, *J. Biol. Chem.*, 153, 207 (1944).

A. Procedure

(a) *Guanidinium-N₃¹⁵ Bromide*. Ammonia-N¹⁵ is prepared from 4.77 g. (0.09 mole) of ammonium-N¹⁵ chloride, special care being taken to ex-

clude moisture.¹ The ammonia-N¹⁵ is passed into a Dry Ice-cooled bomb tube containing 20 ml. of absolute ethanol (Note 1). When the absorption of ammonia-N¹⁵ in the alcohol is complete, the bomb tube is warmed to 0° and kept in an ice-bath. A solution of 3.18 g. (0.03 mole) of freshly prepared cyanogen bromide in 5 ml. of absolute ethanol is added; the tube is sealed and heated to 105–110° for 96 hours (Note 2).

The tube is cooled in ice, opened with a straight break, if possible, and the open end is fire-polished. The gas inlet tube is inserted, and excess ammonia is carried into an acid trap by a slow stream of nitrogen (Note 3). The clear alcoholic solution is evaporated to dryness, and the residue is dried *in vacuo* at room temperature. The yield of guanidinium-N₃¹⁵ bromide is 4.02 g. Although not analytically pure, it is quite satisfactory for the following condensation.

(b) *2,6-Diamino-4-pyrimidinol-1,2,3-N₃¹⁵ Sulfate*. From 5.21 g. of guanidinium-N₃¹⁵ bromide and a 50% excess of ethyl cyanoacetate is prepared 4.08 g. of the diaminopyrimidinol sulfate according to the procedure of Traube.² The guanidine salt is dissolved in a minimum of methyl alcohol and mixed with a solution of sodium methoxide containing 2 moles of sodium per mole of salt. To the solution of free guanidine is added the ethyl cyanoacetate. The solution soon warms considerably, and the separation of needle-like crystals begins. After 5 or 6 hours, the almost pure (cyanoacetyl)guanidine is collected, and evaporation of the filtrate leaves 2,6-diamino-4-pyrimidinol which is recrystallized from hot water.

To convert the (cyanoacetyl)guanidine to the pyrimidine it is treated with a hot, dilute aqueous sodium hydroxide solution which is heated quickly to boiling, then cooled and treated with sulfuric acid in slight excess. The pyrimidine sulfate, which is slightly soluble in cold water, precipitates.

The pyrimidine sulfate is obtained directly from the initial reaction if the residue from the filtrate is recrystallized from dilute sulfuric acid. The sulfate crystallizes with one molecule of water.

(c) *2,6-Diamino-5-nitroso-4-pyrimidinol-1,2,3-N₃¹⁵*. This compound is prepared as described by Traube.³ The diaminopyrimidinol sulfate, 3.45 g., is dissolved in boiling water and treated with an excess of sodium nitrite solution. Immediately, the rose-red nitroso compound is formed in microscopic needles which are almost chemically pure. The yield is 4.08 g. (99.8%).

(d) *2,5,6-Triamino-4-pyrimidinol-1,2,3-N₃¹⁵*. The original procedure of Traube³ is slightly modified to yield the sulfate directly instead of the free base. The finely powdered isotopic nitroso compound, 3.45 g., is slowly added to 50 ml. of boiling water containing 15 ml. of commercial ammonium sulfide. This suspension is kept boiling for 3 hours with occasional stirring and addition of 3 to 5 ml. of ammonium sulfide and the necessary amount of water to keep the volume constant. When all the

nitroso compound is reduced, the solution is immediately filtered (Note 4), with vacuum, directly into 75 ml. of 3 *N* sulfuric acid cooled in ice. Light yellow crystals of 2,5,6-triamino-4-pyrimidinol-1,2,3- N_3^{15} sulfate are thus obtained. The yield is 4.68 g. (82%) of material sufficiently pure for the next reaction.

(e) *Guanine-1,2,3- N_3^{15} Sulfate*, [2-Amino-6(1H)-purinone-1,2,3- N_3^{15} Sulfate]. The isotopic triaminopyrimidinol sulfate, 4.62 g., is heated under reflux for 8 hours with 4.0 g. of anhydrous sodium formate in 50 ml. of 90% formic acid. The solution is filtered and evaporated on the steam-bath. The residue is dissolved in dilute sulfuric acid and boiled with charcoal for 5 hours, and the product is crystallized by cooling the filtrate. The mother liquor is neutralized to pH 5. The precipitated free base is removed by filtration and redissolved in a smaller volume of dilute sulfuric acid, and the guanine again is crystallized as the sulfate salt. Upon repetition of this procedure a total of 2.94 g. of guanine-1,2,3- N_3^{15} sulfate is obtained (Note 5).

(f) *Xanthine-1,3- N_2^{15}* , [2,6(1H,3H)-Purinedione-1,3- N_2^{15}]. Guanine-1,2,3- N_3^{15} sulfate, 0.032 g., is dissolved in 2.0 ml. of 4 *N* sulfuric acid. The solution is heated in a water-bath, 0.024 g. of sodium nitrite is added in small portions, and the solution is neutralized and cooled in an ice-bath. The dark colored xanthine-1,3- N_2^{15} which precipitates is purified by formation of its silver salt, which is then decomposed with dilute hydrochloric acid. On neutralization to pH 5 xanthine-1,3- N_2^{15} crystallizes in analytically pure form.

B. Notes

1. A gas inlet tube, especially constructed to fit the bomb tube, is used. An exit tube carries any ammonia that escapes into a trap containing dilute sulfuric acid.

2. The length of time necessary was determined with nonisotopic ammonia. At the end of 96 hours at 110°, the test for cyanamide with ammoniacal silver nitrate was consistently negative.

3. From 94 to 96% of the excess ammonia is collected.

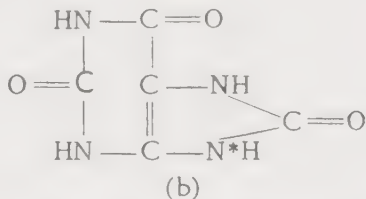
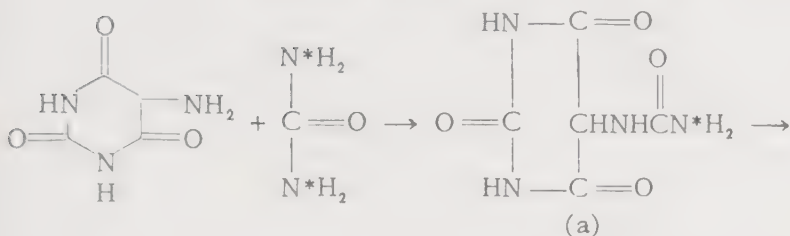
4. On prolonged boiling in the absence of excess ammonium sulfide the solution turns pink. This is not prevented by use of an atmosphere of nitrogen.

5. The molecular formula is $(C_5H_5N_5O)_2 \cdot H_2SO_4 \cdot 2H_2O$.

¹See cyanamide- $N_{1/2}^{15}$.

²W. Traube, German patent 134,984 (1903).

³W. Traube, Ber., 33, 1371 (1900).

URIC-9-N¹⁵ ACID[2,6,8(1H,3H,9H)-Purinetriione-9-N¹⁵]

L. F. Cavalieri, V. E. Blair and G. B. Brown, J. Am. Chem. Soc., 70, 1240 (1948).

A. Procedure

(a) *Pseudouric-9-N¹⁵ Acid*. Urea-N₂¹⁵, 0.55 g. (0.0092 mole), is fused with 0.5 g. (0.0035 mole) of uramil¹ at 150–170° for 45 minutes. The cooled melt is extracted with 20 ml. of boiling water, and the insoluble material is filtered off. The filtrate is decolorized with carbon and evaporated to about 6 ml. Upon cooling, 0.355 g. (50% yield) of ammonium pseudourate-9-N¹⁵ precipitates. This material is dissolved in aqueous sodium hydroxide, and pseudouric-9-N¹⁵ acid is precipitated by acidifying with hydrochloric acid; yield, 0.20 g. (35%).

(b) *Uric-9-N¹⁵ Acid*, (2,6,8(1H,3H,9H)-Purinetriione-9-N¹⁵). The pseudouric-9-N¹⁵ acid is converted to uric-9-N¹⁵ acid by heating for 15–20 minutes with a 500-fold amount of 20% hydrochloric acid. The solution is evaporated to 1/15 its volume and diluted with water, and the crystalline product is collected on a filter. The uric-9-N¹⁵ acid (Note 1) is recrystallized from water.

B. Notes

1. The urea-N₂¹⁵ contained 0.97 atom per cent excess N¹⁵. Both the pseudouric acid and the uric acid contained 0.28 atom per cent excess N¹⁵, which is slightly more than the theoretical 0.24 atom per cent excess N¹⁵ for the introduction of one nitrogen atom from the urea. Since in the analogous reaction² of 5,6-diamino-2,4-pyrimidinediol with urea-N₂¹⁵ both of the amino groups of the urea were eliminated and no isotopic nitrogen was introduced into the uric acid formed, it may be presumed that, in the uric acid formed from urea-N₂¹⁵ and uramil, the isotopic nitrogen was in-

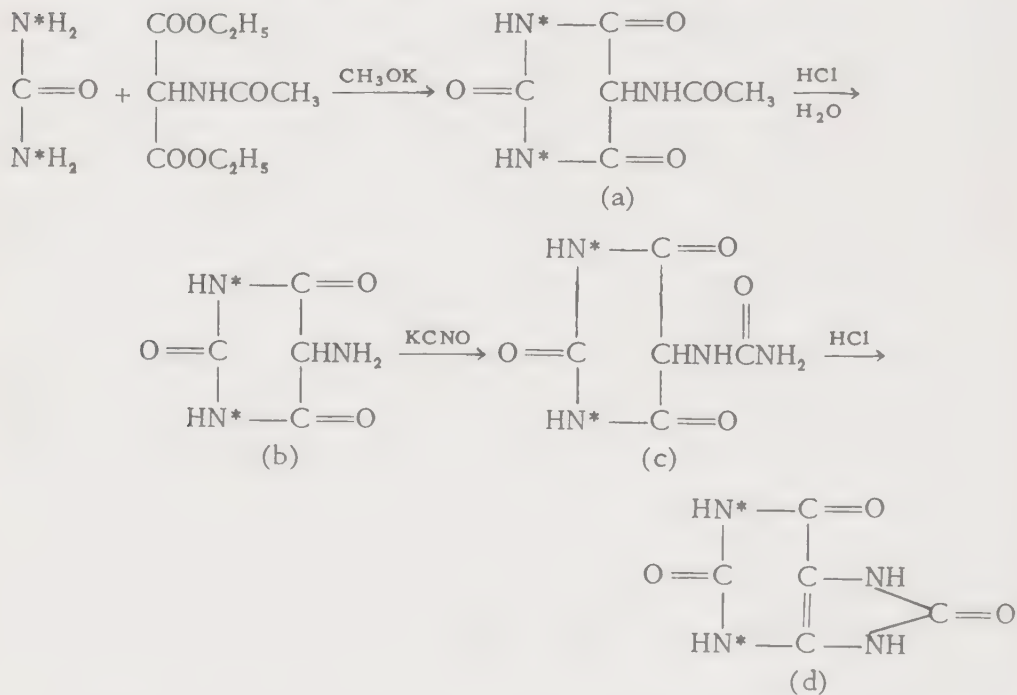
troduced chiefly in position 9. Clusius and Vecchi³ obtained the same result with urea- N_2^{15} and 5,6-diamino-2,4-pyrimidinediol; isotopic nitrogen was not introduced into the resulting uric acid.

¹*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1943, p. 617.

²P. A. Levene and J. K. Senior, *J. Biol. Chem.*, 25, 618 (1916).

³K. Clusius and M. Vecchi, *Helv. Chim. Acta*, 36, 1324 (1953).

URIC-1,3- N_2^{15} ACID
[2,6,8(1H,3H,9H)-Purinetrione-1,3- N_2^{15}]



L. F. Cavalieri, V. E. Blair and G. B. Brown, *J. Am. Chem. Soc.*, 70, 1240 (1948).

A. Procedure

(a) *5-Acetamidobarbituric-1,3- N_2^{15} Acid*, (*Acetyluramil-1,3- N_2^{15}*). A three-necked flask is equipped with a Hershberg stirrer, reflux condenser and glass stopper. The apparatus is dried, and potassium methylate is prepared by adding absolute methanol to 0.9 g. (0.23 mole) of clean potassium under benzene. The solvents are removed under reduced pressure, and to the dry residue is added 1.180 g. (0.0197 mole) of urea- N_2^{15} in 4 ml. of absolute methanol. Ethyl acetamidomalonate,¹ 4.3 g. (0.02 mole), is dissolved in 8 ml. of methanol and added in one portion to the urea solution. The mixture is heated under reflux and stirred for 4 hours. The white precipitate of potassium acetyluramil-1,3- N_2^{15} is collected by filtration; yield, 3.74 g. (85%).

(b) 5-Aminobarbituric-1,3- N_2^{15} Acid, (Uramil-1,3- N_2^{15}). The potassium acetyluramil-1,3- N_2^{15} (3.61 g., 0.0161 mole) is hydrolyzed to uramil in 30 ml. of concentrated hydrochloric acid. The mixture is heated to boiling for about five minutes at which time all the solid is in solution. The uramil-1,3- N_2^{15} is precipitated by diluting the solution with 70 ml. of water; yield, 2.16 g. (94%).

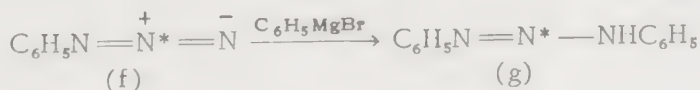
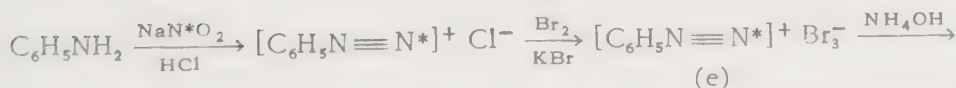
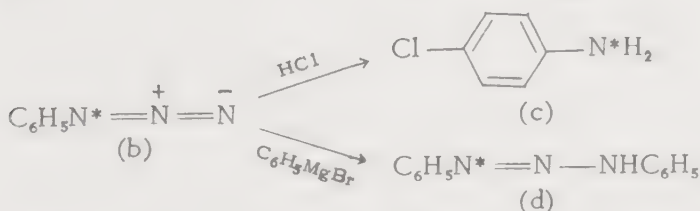
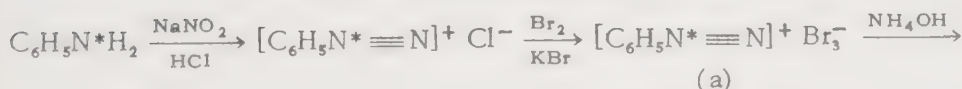
(c) Potassium Pseudourate-1,3- N_2^{15} . Uramil-1,3- N_2^{15} , 2.16 g. (0.0151 mole), is added in one portion to a boiling solution of 55 g. of potassium cyanate in 260 ml. of water. The uramil dissolves, producing a clear pink solution, and potassium pseudourate-1,3- N_2^{15} precipitates almost immediately. The solution is heated under reflux for one-half hour with stirring. Upon cooling the solution, 3.14 g. (92%) of product is obtained.

(d) 2,6,8(1H, 3H, 9H)-Purintrione-1,3- N_2^{15} (Uric-1,3- N_2^{15} Acid). Potassium pseudourate-1,3- N_2^{15} is converted to uric-1,3- N_2^{15} acid according to the procedure of Fischer.² The finely powdered salt is heated for 15-20 minutes with a 500-fold amount of 20% hydrochloric acid. The solution is evaporated to 1/15 of its volume, and most of the uric acid crystallizes before cooling. The liquor is diluted with water and filtered. Yields of 72 to 80% are obtained, after two recrystallizations.

¹H. R. Snyder and C. W. Smith, J. Am. Chem. Soc., 66, 350 (1944).

²E. Fischer, Ber., 30, 559 (1897).

1,3-DIPHENYLTRIAZENE-1,3- $N_{1/2}^{15}$



K. Clusius and H. Hürzeler, Helv. Chim. Acta, 37, 383 (1954).

A. Procedure

(a) Benzenediazonium- λ - N^{15} Perbromide (Note 1). An ice-cold solution

of 1.40 g. (15 mmoles) of aniline- N^{15} in 10 ml. of 19% hydrochloric acid is treated with a solution of 1.04 g. of sodium nitrite in 5 ml. of water. To the rapidly stirred benzenediazonium- α - N^{15} chloride solution, cooled in ice, is added dropwise a solution of 0.95 ml. of bromine in 9.5 ml. of 25% potassium bromide solution; any excess of the latter is avoided. The orange-yellow flocculent precipitate of the perbromide is collected, washed with a little alcohol and with ether, and dried in a stream of air. The yield is 4.05 g. (78% based on aniline- N^{15}).

(b) *Azidobenzene-1- N^{15}* . To a mixture of 15 ml. of 21% ammonium hydroxide and 10 ml. of ether, which is stirred rapidly and cooled in ice, is added 4.05 g. of finely powdered benzenediazonium- α - N^{15} perbromide in small portions. As the perbromide sinks through the ether layer it reacts rapidly and completely with the ammonium hydroxide, and the resulting azidobenzene-1- N^{15} dissolves in the ether layer (Note 2). After removal of ether, the resulting two-phase reaction product is steam-distilled. The ammoniacal distillate is acidified with dilute sulfuric acid, and the azidobenzene-1- N^{15} is extracted with ether and dried over potassium hydroxide. After the ether is evaporated, the product is distilled under high vacuum at room temperature into a dry flask cooled with Dry Ice (Note 3). The yield is 775 mg. (55%).

(c) *4-Chloroaniline- N^{15}* . Using a vacuum apparatus (Note 4), a mixture of 5 ml. of 19% hydrochloric acid and 147 mg. of azidobenzene-1- N^{15} is freed of air by twice freezing the mixture and evacuating the system. The flask is then filled with pure carbon dioxide (Note 5) and heated at a temperature of 120–130° for 45 minutes. After evaporation of the reaction mixture, the residue of 4-chloroaniline- N^{15} is purified by sublimation under vacuum (Note 6).

(d) *1,3-Diphenyltriazene-1,3- $N^{15}_{1/2}$* . According to the procedure of Dimroth,¹ 620 mg. of azidobenzene-1- N^{15} is added dropwise to an ether solution of phenylmagnesium bromide, in a flask equipped with an efficient reflux condenser (Note 7). The suspension of the intermediate is treated with ice-water to decompose the orange-colored magnesium compound. The basic magnesium salt is made to dissolve by the addition of ammonium chloride, and the ether layer is separated and dried over sodium sulfate. By dilution of the ether solution with an excess of bromobenzene, the product is obtained in a nearly pure state. The yield of product after recrystallization from ligroin is 355 mg.; m.p. 97°.

Starting with ordinary aniline and sodium nitrite- N^{15} and employing the reactions described above, the following isotopic compounds are also prepared (Note 8).

(e) *Benzenediazonium- β - N^{15} Perbromide*.

(f) *Azidobenzene-2- N^{15}* .

(g) *1,3-Diphenyltriazene-2- N^{15}* .

B. Notes

1. The procedure, a modification of that described by Gattermann and Wieland,² avoids precipitation of the product as an oil. Preparation of azidobenzene-1-N¹⁵ via the diazonium perbromide avoids the formation of isotopic isomers.

2. In this improvement on the procedure of Griess,³ the formation of a sticky oil, which hinders working up of the product, is avoided.

3. Phenyl azide explodes when rapidly heated but may be distilled at pressures of 5 mm. or less. It is advisable to use as low a bath temperature as possible; the b.p. at 5 mm. is 49-50°.⁴

4. This apparatus was similar to that described earlier⁵ for the oxidation of hydrazoic-1-N¹⁵ acid; a diagram was given.

5. The carbon dioxide, prepared by heating sodium bicarbonate, was twice sublimed under vacuum to free it of traces of air.

6. 4-Chloroaniline, m.p. 70-71°, boils at 232° and is soluble in hot water and quite soluble in alcohol, ether, acetone and carbon disulfide.

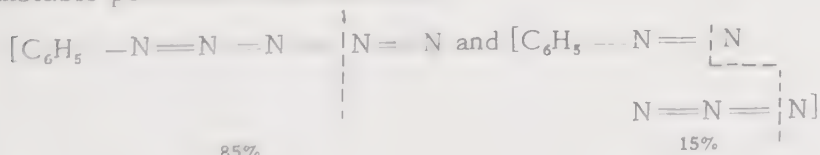
7. It may be necessary to moderate the reaction by cooling the flask.

8. Isotopic analysis of the various intermediate and end products, including aniline and ammonia from the reduction of diphenyltriazene, demonstrated that the nitrogen atoms in phenyl azide can only be linear and are not arranged in a three-membered ring as suggested by Curtius.⁶

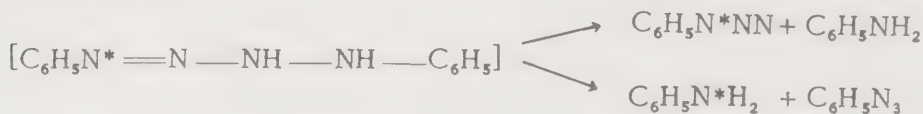
C. Other Preparations

Phenyl azide labeled with nitrogen-15 has been prepared from ordinary phenylhydrazine and nitrous-N¹⁵ acid.⁷ It was found that the reaction proceeded by at least two paths since the mixture of compounds designated by the name azidobenzene-2,3-N¹⁵ was obtained. The percentage of the two isotopic isomers depended greatly upon the temperature of the diazotization; at 0-2° less than 2% of azidobenzene-2-N¹⁵ was found and at 10-13° less than 7%. Treatment of the former compound with phenylmagnesium bromide gave 1,3-diphenyltriazene-1,2-N¹⁵₂.

In another mechanism study,⁸ azidobenzene-1-N¹⁵, azidobenzene-2-N¹⁵ and azidobenzene-2,3-N¹⁵₂ have been prepared from, respectively: benzenediazonium- α -N¹⁵ ion with azide ion, benzenediazonium- β -N¹⁵ ion with azide ion, and benzenediazonium ion with azide-1-N¹⁵ ion. The expected mechanism in which nitrogen of diazonium ion would be replaced by the azide ion, as in the formation of aryl halides from diazonium salts, was not realized. The reaction probably proceeds by two paths through unstable pentazene intermediates:



Azidobenzene-1- N^{15} has also been prepared⁹ from benzenediazonium- α - N^{15} ion and phenylhydrazine in a mineral acid medium. This reaction probably proceeds through a tetrazene intermediate in two ways, as in the equation below.



¹O. Dimroth, *Ber.*, 36, 909 (1903).

²L. Gattermann and H. Wieland, *Laboratory Methods of Organic Chemistry*, Macmillan and Co., London, 1941, p. 289.

³P. Griess, *Ann.*, 137, 65 (1866).

⁴*Organic Syntheses*, Vol. 22, Wiley, New York, 1942, p. 96.

⁵K. Clusius and H. Hürzeler, *Helv. Chim. Acta*, 36, 1326 (1953).

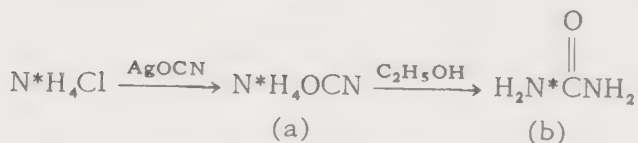
⁶Th. Curtius, *Ber.*, 23, 3023 (1890).

⁷K. Clusius and H. R. Weisser, *Helv. Chim. Acta*, 35, 1548 (1952).

⁸K. Clusius and H. Hürzeler, *ibid.*, 37, 798 (1954).

⁹K. Clusius and H. Craubner, *ibid.*, 38, 1060 (1955).

UREA- N_1^{15}



D. L. Williams, U. S. Atomic Energy Comm. Report, AECU-664; *Nuclear Sci. Abstr.*, 4, 227 (1950).

A. Procedure

(a) *Ammonium- N^{15} Cyanate*. In a 40-ml. centrifuge tube are thoroughly mixed 0.1535 g. (0.00287 mole) of ammonium- N^{15} chloride, 0.6105 g. (0.00407 mole) of silver cyanate (40% excess), and 10 ml. of distilled water. The mixture is stirred rapidly for 7 hours (Note 1) and then filtered. The filtrate, containing a trace of silver cyanate (Note 2), is transferred to a 250-ml. flask. The precipitate of silver chloride and silver cyanate is washed with a total of 100 ml. of absolute ethanol (Note 3) which is added to the filtrate.

(b) *Urea- N_1^{15}* . The flask containing the alcohol solution of ammonium- N^{15} cyanate is tightly stoppered and heated in an oil-bath for 16.5 hours at 55° (Note 4). The alcoholic solution is evaporated to dryness, and the residue is extracted with a total of 40 ml. of absolute methanol. The extract is transferred into a vacuum sublimator and evaporated to dryness. The product is sublimed during two 3-hour periods at a bath temperature of 68–70° and a pressure of 0.002–0.003 mm. (Note 5). The product, washed from the sublimator with methanol and evaporated to

dryness, weighs 0.1465 g. (85.4%) and melts at 131-132°. This material, recrystallized from 20 ml. of acetone, melts at 132-133°.

B. Notes

1. The unreacted silver cyanate and the silver chloride formed are kept in suspension and finely divided.

2. The solubility of silver cyanate in water is 0.072 g. per liter at 18°.

3. Walker and Kay¹ have reported that no carbonate is formed when the solvent is 70 to 90% ethanol.

4. Only a slight test for cyanate ion is given by the solution after 5 hours of heating.

5. Contrary to statements in the early literature, urea can be sublimed under high vacuum without the formation of ammonium carbonate. It is advantageous to interrupt the sublimation after 3 hours, redissolve the residue in methanol, and again evaporate the solvent, thus redistributing the residue and remaining product.

C. Other Preparations

Urea-N₁¹⁵ has been prepared,² in excellent yield, from aqueous-alcoholic solution of ammonium-N¹⁵ sulfate and potassium cyanate at room temperature.

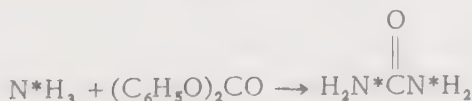
Urea-C¹⁴-N₁¹⁵ has been prepared³ in 41.7% yield by heating ammonium-N¹⁵ cyanate-C¹⁴, prepared from silver cyanate-C¹⁴ and ammonium-N¹⁵ chloride, in 90% alcohol.

¹J. Walker and S. A. Kay, J. Chem. Soc., 71, 489 (1897).

²H. B. van Dyke, J. V. Scudi and D. L. Tabern, J. Pharmacol. Exptl. Therap., 90, 264 (1947).

³D. L. Williams and A. R. Ronzio, J. Am. Chem. Soc., 74, 2407 (1952).

UREA-N₂¹⁵



L. F. Cavalieri, V. E. Blair and G. B. Brown, J. Am. Chem. Soc., 70, 1240 (1948).

A. Procedure

This preparation of urea-N₂¹⁵ from ammonia-N¹⁵ and phenyl carbonate is a modification of the procedure of Bloch and Schoenheimer,¹ who added the use of copper powder as catalyst to the method of Hentschel.²

A three-necked flask is equipped with a reflux condenser, dropping funnel and an inlet tube for nitrogen. A potassium hydroxide drying tube is placed on top of the condenser, and a wide-mouth tube from this extends into a glass bomb tube, 15 cm. above the bottom. An outlet tube from the bomb is passed through an acid trap containing a known quantity of standard acid. A bath of liquid nitrogen is placed around the bomb.

A solution of 4.088 g. (0.0511 mole) of ammonium-N¹⁵ nitrate in 25 cc. of water is introduced into the flask. The bomb tube is charged with an intimate mixture of 4.95 g. (0.0231 mole) of phenyl carbonate and 0.13 g. of copper powder. Before the ammonia-N¹⁵ is liberated from the ammonium-N¹⁵ nitrate, the apparatus is swept with nitrogen for 10 minutes. The bomb tube is then immersed in liquid nitrogen. While continuing the slow stream of nitrogen, an excess of aqueous sodium hydroxide is slowly introduced, and the solution is heated to boiling (Note 1). The flask is heated for 3 hours (Note 2); then the bomb tube is transferred to a Dry Ice-bath and sealed. The closed tube is heated in a bath at 90–100° for 4 hours (Note 3). The contents of the tube are dissolved in a minimal amount of warm water (about 50 ml.), and the copper powder is removed by filtration. The filtrate is extracted with six 50-ml. portions of chloroform, and the aqueous layer is decolorized and evaporated to dryness on a water-bath. Recrystallization of the crude urea, 0.977 g., from acetone gives 0.830 g. (57% based on ammonia condensed) of urea-N₂¹⁵; m.p. 133–135° (stage).

B. Notes

1. Care must be exercised not to liberate the ammonia too rapidly since the inlet tube inside the bomb may become clogged with solid ammonia.

2. About 5% of uncondensed ammonia is collected in the acid trap.

3. Buzard and Bishop³, while using this procedure, have experienced a number of explosions when the tube is warmed. They have suggested explanations, such as, the condensation of nitrogen in the tube and formation of an unstable compound between nitrogen and ammonia. Cavalieri and Brown⁴ have suggested that these explosions are the result of condensation of oxygen, by liquid nitrogen, from the tank nitrogen used in sweeping the ammonia into the bomb tube.

C. Other Preparations

Using a procedure similar to that described, Leitch,⁵ has reported the synthesis of urea-N₂¹⁵, in approximately 90% yield, from ammonium-N¹⁵ nitrate and phenyl carbonate.

¹K. Bloch and R. Schoenheimer, *J. Biol. Chem.*, 138, 186 (1941).

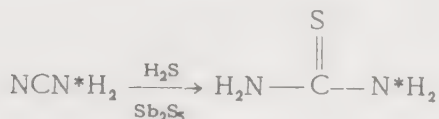
²W. Hentschel, *Ber.*, 17, 1284 (1884).

³J. Buzard and C. Bishop, *J. Am. Chem. Soc.*, 74, 2925 (1952).

⁴L. F. Cavalieri and G. B. Brown, private communication.

⁵L. C. Leitch and W. M. Davidson, *Sci. Agr.*, 29, 189 (1949).

THIOUREA-N¹⁵



A. A. Plentl and R. Schoenheimer, *J. Biol. Chem.*, 153, 210 (1944).

Procedure

Cyanamide-N¹⁵, 2.66 g., is dissolved in 70 ml. of water, and 5.34 g. of antimony pentasulfide and 0.7 ml. of concentrated hydrochloric acid are added. The mixture is heated to 90° on a steam-bath, and a rapid stream of hydrogen sulfide is passed through the suspension for 2 hours. After removal of the excess hydrogen sulfide by boiling the solution, the antimony pentasulfide is collected and washed with hot water. The filtrate is treated with 0.2 g. of potassium carbonate and filtered through a small amount of infusorial earth. It is then evaporated to dryness under reduced pressure, and the residue is recrystallized from propyl alcohol until its melting point is 174°. The total yield, part of which is obtained by concentration of the mother liquors, is 2.4 g. of thiourea-N¹⁵.

AMMONIA-N¹⁵



R. Schoenheimer and S. Ratner, *J. Biol. Chem.*, 127, 301 (1939).

A. Procedure

Ammonia-N¹⁵ is generated from an ammonium-N¹⁵ salt in a modified Claisen flask containing 10 N sodium hydroxide solution. The vertical side-arm is fitted with a condenser (also vertical) to prevent distillation of water. The neck of the flask is equipped with a dropping funnel and gas inlet tube. The aqueous sodium hydroxide solution is heated to gentle boiling, and a concentrated solution of the ammonium-N¹⁵ salt is added slowly from the dropping funnel. With a slow stream of nitrogen bubbling through the solution, heating is continued until all the ammonia is swept over into a suitable cold trap or absorbing medium.

B. Other Preparations

On a number of occasions,¹⁻⁴ ammonia-N¹⁵ has been generated from an ammonium-N¹⁵ salt by adding concentrated sodium hydroxide and heating

the solution under reflux. Stetten⁵ dropped ammonium-N¹⁵ nitrate solution into hot concentrated sodium hydroxide solution, and aerated the ammonia-N¹⁵ into the reaction mixture. A similar procedure was used by Stetten and Schoenheimer.⁶

¹W. S. Fones and J. White, *Arch. Biochem.*, **20**, 118 (1949).

²A. Bendich, J. G. Tinker and G. B. Brown, *J. Am. Chem. Soc.*, **70**, 3109 (1948).

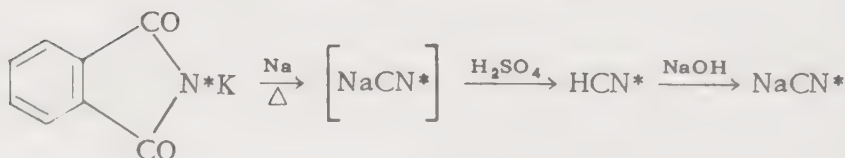
³L. F. Cavalieri, V. E. Blair and G. B. Brown, *ibid.*, **70**, 1240 (1948).

⁴L. F. Cavalieri, J. F. Tinker and A. Bendich, *ibid.*, **71**, 533 (1949).

⁵M. R. Stetten, *J. Biol. Chem.*, **181**, 34 (1949).

⁶M. R. Stetten and R. Schoenheimer, *ibid.*, **153**, 115 (1944).

SODIUM CYANIDE-N¹⁵



A. G. MacDiarmid and N. F. Hall, *J. Am. Chem. Soc.*, **75**, 4850 (1953).

A. Procedure

A small steel bomb is made by drilling a hole, $2\frac{1}{2} \times \frac{3}{8}$ inches, in a hexagonal steel bar. The bomb is sealed by a bolt which screws into the hole for about half an inch, and the plug is made gas-tight by means of a soft copper washer. The bomb is thoroughly washed with soap and hot water, rinsed with water and acetone, and dried before use (Note 1).

Potassium phthalimide-N¹⁵, 0.3 g., and 1.5 g. of sodium slices are placed in the bomb, which is then heated in a furnace in an upright position for 20 minutes at 700°. After cooling the bomb, water is carefully added dropwise to the contents to decompose any remaining sodium. The carbonized mass is then extracted with hot water. The solution is treated with 5 ml. of 0.1 N barium hydroxide, then heated to boiling, and filtered to remove carbon and barium carbonate. The filtrate is acidified with 50% sulfuric acid, and the hydrogen cyanide-N¹⁵ is distilled into 20 ml. of 0.1 N sodium hydroxide (Note 2).

B. Notes

1. Traces of rust cause the formation of hexacyanoferrate (II) impurity in the sodium cyanide.

2. Ordinary sodium cyanide may then be added to this solution to obtain a desired normality or atom per cent excess N¹⁵. Yields of 97–100% were obtained in four preparations using ordinary potassium phthalimide.

SODIUM THIOCYANATE-N¹⁵

C. Tesar and D. Rittenberg, *J. Biol. Chem.*, 170, 36 (1947).

A. Procedure (Note 1)

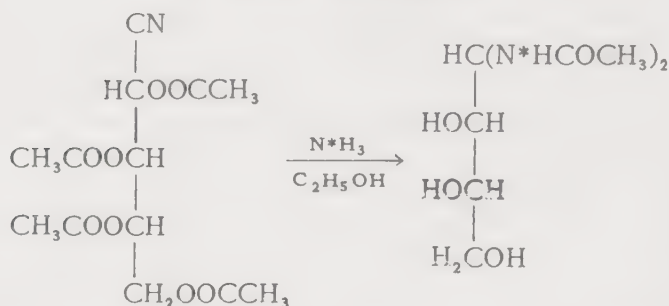
To a mixture of 25 ml. of carbon disulfide and 17 g. of ferric hydroxide in 40 ml. of absolute methanol, 10.8 g. of ammonium-N¹⁵ nitrate is added, and the mixture is shaken mechanically in a tightly closed bottle. Sodium hydroxide pellets, in nine portions totaling 10.8 g., are added at 2-hour intervals. After the mixture is shaken for an additional 24 hours, it is diluted with water and centrifuged. The precipitate is washed several times by centrifugation. The supernatant liquors are combined, saturated with hydrogen sulfide, filtered, acidified to litmus with hydrochloric acid, and heated to boiling. The cooled solution is neutralized by the addition of dilute sodium hydroxide (Note 2) and evaporated to dryness. The salts are extracted with several portions of absolute ethanol, and the extract, containing the sodium thiocyanate, is evaporated on the steam-bath. The residue is extracted with 100 ml. of absolute ethanol, and the extract is evaporated to dryness to obtain 9.8 g. (90%) of sodium thiocyanate-N¹⁵ (Note 3).

B. Notes

1. The procedure of Schulze¹ was modified to conserve isotopic ammonia.
2. An amount equivalent to the hydrochloric acid added is used.
3. The product is 99% pure as assayed colorimetrically against standardized normal thiocyanate.

¹J. Schulze, *J. prakt. Chem.*, 27, 518 (1883).

1,1-DIACETAMIDO-1-DEOXY-L-ERYTHROSE-N¹⁵
(L-Erythrose Diacetamide-N¹⁵)



R. C. Hockett, V. Deulofeu and J. O. Deferrari, *J. Am. Chem. Soc.*, **72**, 1840 (1950).

A. Procedure

When a fully acetylated aldonic acid nitrile is treated with aqueous ammonia, the main product is usually an open chain diacetamide derivative of the aldose sugar having one less carbon atom.^{1,2,3}

One mole of L-arabononitrile tetraacetate is reacted with ethanolic ammonia-N¹⁵ (6.2 atom per cent excess N¹⁵) in the presence of 4 to 8 moles of normal acetamide (Note 1). The L-erythrose diacetamide formed contains 5.69–5.71 atom per cent excess N¹⁵ (Note 2).

B. Notes

1. This experiment was designed to test the reaction mechanism proposed by Isbell and Frush.⁴ They suggested that an acetylated aldehyde sugar (produced by hydrolysis of the nitrile) may first add ammonia at the aldehyde group and that acetyl may then migrate to nitrogen from an adjacent ester group to produce the acetamide-like structure. The excess acetamide is, then, unnecessary to the reaction. This proved to be the case.

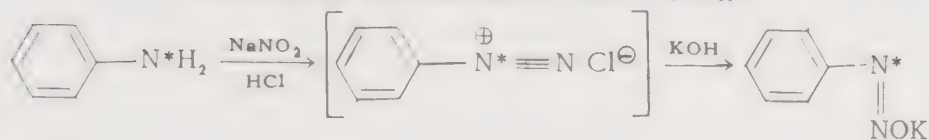
2. In a control experiment, 0.1 g. of acetamide was dissolved in ethanolic ammonia-N¹⁵ containing 6.2 atom per cent excess N¹⁵. After 48 hours, evaporation of the ethanol yielded acetamide containing no excess N¹⁵. Therefore, no detectable exchange of N¹⁵ for normal nitrogen takes place under these conditions.

¹L. Maquenne, *Comp. rend.*, **130**, 1402 (1900).

²A. Wohl, *Ber.*, **26**, 730 (1893).

³R. C. Hockett and L. B. Chandler, *J. Am. Chem. Soc.*, **66**, 957 (1944).

⁴H. S. Isbell and H. L. Frush, *J. Am. Chem. Soc.*, **71**, 1579 (1949).

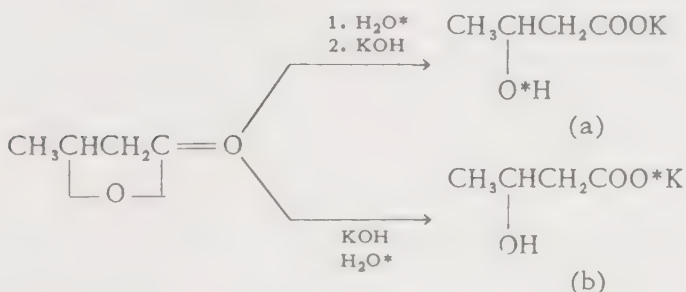
POTASSIUM BENZENEISODIAZOATE- α -N¹⁵

G. A. Swan and P. Kelly, J. Chem. Soc., 1954, 416.

Procedure

A mixture of 1.5 g. of potassium hydroxide and 0.5 ml. of water is heated in a nickel crucible until homogeneous and then cooled in ice with stirring. To the potassium hydroxide is added, with stirring, a solution of benzenediazonium- α -N¹⁵ chloride, prepared from 0.26 g. of aniline-N¹⁵ in 0.5 ml. of water and 0.35 ml. of concentrated hydrochloric acid. When dissolution is complete, the crucible is heated in an oil-bath at 140°, and the contents are stirred until the salt solidifies. The crucible is then cooled to 100°; the product is dissolved in hot water (1/2 volume), and the solution is cooled. The resulting solid is collected on a sintered glass funnel and extracted with a small volume of ethanol at 40°. The filtered solution is diluted with ether and cooled to 0°. The yield of potassium benzeneisodiazotate- α -N¹⁵, which is washed with ether and dried under vacuum, is 0.13 g.

OXYGEN-18 COMPOUNDS

POTASSIUM 3-HYDROXYBUTYRATE-1-O¹⁸

A. R. Olson and J. L. Hyde, J. Am. Chem. Soc., 63, 2459 (1941).

A. Procedure

(a) *Potassium 3-Hydroxybutyrate-3-O¹⁸*. β -Butyrolactone, 0.40 ml. (4.9 mmoles), is dissolved in 10.0 ml. of water-O¹⁸ of known isotopic content. This solution is kept at 25° until hydrolysis is at least 99% complete, as calculated from rate constants¹ (Note 1). After the hydrolysis period, the solution is neutralized to phenolphthalein end point with potassium hydroxide and then evaporated *in vacuo*. The solid residue is warmed for 3 hours on a water-bath, while under high vacuum (Note 2).

(b) *Potassium 3-Hydroxybutyrate-1-O¹⁸*. This compound is prepared according to the above procedure but with an aqueous medium of 1 N potassium hydroxide solution in water-O¹⁸.

B. Notes

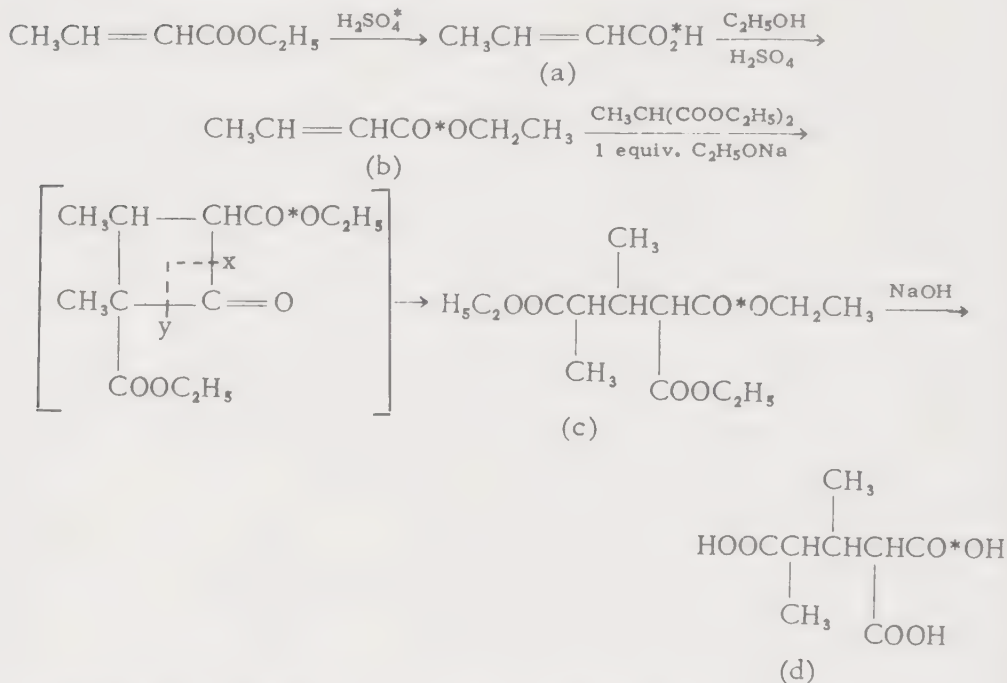
1. The change in pH, determined in a parallel experiment, was from 5 to 2.2.

2. The products were analyzed for excess oxygen-18 in the 3-hydroxyl group by dehydration at 200°. The resulting water was equilibrated with carbon dioxide which was then analyzed in a mass spectrometer. The results indicated that in the hydrolysis with water only, the fission of an

alkyl-oxygen bond occurs; in the 1 *N* potassium hydroxide solution, the carbonyl-oxygen bond is broken.

¹A. R. Olson and R. J. Miller, *J. Am. Chem. Soc.*, 60, 2687 (1938).

2-CARBOXY-3,4-DIMETHYLGLUTARIC-1-O¹⁸ ACID



D. Samuel and D. Ginsburg, *J. Chem. Soc.*, 1955, 1288.

A. Procedure

(a) *Crotonic-O₂¹⁸ Acid*, (2-*Butenoic-O₂¹⁸ Acid*). Ethyl crotonate, 30 g., is refluxed with 30 ml. of 1 *N* sulfuric acid in water-O¹⁸. The aqueous suspension is then extracted three times with ether; the combined extract is washed with water and dried over sodium sulfate. Upon removal of the ether, colorless crystals of crotonic-O₂¹⁸ acid, m.p. 74°, are obtained (Note 1).

(b) *5-Oxa-2-hepten-4-one-4-O¹⁸*, (Ethyl O₁¹⁸-Crotonate). The above free acid is dissolved in 120 ml. of absolute ethanol which contains 1.2 ml. of concentrated sulfuric acid. After 24 hours at room temperature, the solution is added to about 600 ml. of water, according to the procedure of Michael,¹ and the mixture is thoroughly shaken. The oil which separates is washed four times with small amounts of water. Distillation of the crude ester affords 60 g. (75%) of ethyl O₁¹⁸-crotonate, b.p. 143° (Note 2).

(c) *Ethyl 2-Ethoxycarbonyl-O¹⁸-3,4-dimethylglutarate*. According to the following adaptation of the procedure of Michael and Ross,² to a suspen-

sion of 0.05 mole of sodium ethoxide in dry ether is added 8.75 g. (0.05 mole) of ethyl methylmalonate, 5.75 g. (0.05 mole) of ethyl O_1^{18} -crotonate and 75 ml. of ether. The mixture is refluxed for 5 hours; then it is cooled and poured into 25 ml. of water containing 3 g. of acetic acid. The ethereal layer is separated and washed with 10% sodium carbonate and with water. After removal of ether, distillation of the residue yields first unchanged esters, b.p. 60–100° (12 mm.) and then 8.64 g. (60%) of ethyl 2-ethoxycarbonyl- O_1^{18} -3,4-dimethylglutarate, b.p. 148–150° (3 mm.) (Note 3).

(d) *2-Carboxy-3,4-dimethylglutaric-1- O_1^{18} Acid*. The above ester is refluxed with 1 *N* sodium hydroxide to effect hydrolysis. The mixture is then chilled in an ice-bath, covered with ether and acidified by the dropwise addition of hydrochloric acid, with stirring. The ether solution is separated from the aqueous phase and dried. After removal of ether, the free acid is recrystallized from a mixture of light petroleum, chloroform and ether, m.p. 145°.

B. Notes

1. According to Samuel and Ginsburg, in addition to the O^{18} introduced by hydrolysis of the ester, there is also exchange between the free carboxyl groups and the acidic water- O^{18} medium.

2. Michael¹ gave a b.p. of 139° at 771 mm.

3. In the Michael condensation,^{1,7} when ethyl crotonate (an "acceptor" compound having an activated double bond) is treated with ethyl methylmalonate (a "donor" compound having an active α -hydrogen atom) in the presence of 1/6 of an equivalent of sodium ethoxide, the so-called "normal" product is obtained, i.e., ethyl 2-ethoxycarbonyl-2,3-dimethylglutarate. When 1 equivalent of sodium ethoxide is used, the "abnormal" product, ethyl 2-ethoxycarbonyl-3,4-dimethylglutarate, is obtained. The cyclobutanone intermediate, suggested by Holden and Lapworth,⁵ presents a means by which the migration of an ethoxycarbonyl group can occur. If alcoholysis occurs at x, the "normal" product is obtained; if alcoholysis occurs at y the "abnormal" product results. Shafer, Loeb and Johnson⁸ have suggested that the first stage of the process may not be the formation of the new carbon-carbon bond but rather a condensation of the Claisen type between the "acceptor," which has been modified through the addition of ethanol across the double bond, and the carboxylate group of the "donor."

¹A. Michael, Ber., 33, 3766 (1900).

²A. Michael and J. Ross, J. Am. Chem. Soc., 52, 4598 (1930).

³J. F. Thorpe, J. Chem. Soc., 77, 923 (1900).

⁴E. H. Farmer, S. C. Ghosal and G. A. R. Kon, *ibid.*, 1936, 1804.

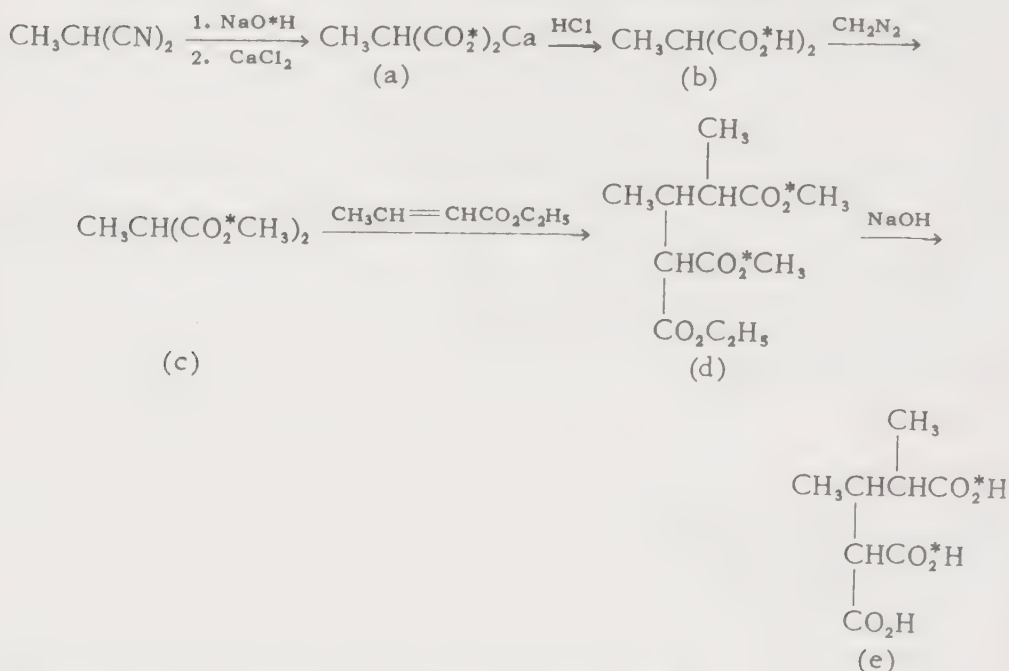
⁵N. E. Holden and A. Lapworth, *ibid.*, 1931, 2368.

⁶A. Michael, J. Org. Chem., 2, 303 (1938).

⁷*Organic Reactions*, Vol. IX, Wiley, New York, 1957, pp. 124, 125, 130.

⁸P. R. Shafer, W. E. Loeb and W. S. Johnson, *J. Am. Chem. Soc.*, 75, 5963 (1953).

2-CARBOXY-3,4-DIMETHYLGUTARIC- O_4^{18} ACID



D. Samuel and D. Ginsburg, *J. Chem. Soc.*, 1955, 1288.

A. Procedure

(a) *Calcium Methylmalonate- O_4^{18}* . A mixture of 2.4 g. of methylmalononitrile (Note 1) and 13 ml. of 5 *N* sodium hydroxide- O^{18} solution (Note 2) is refluxed until the evolution of ammonia ceases. Then, 4 g. of calcium chloride is added to the hot solution. The precipitate of calcium methylmalonate- O_4^{18} is collected and washed alternately with hot water and cold water and finally with ether. The dry salt weighs 4.7 g.

(b) *Methylmalonic- O_4^{18} Acid*. The above dry salt, 4.7 g., is suspended in 10 ml. of dry, ice-cold ether and, with stirring and cooling, 5 ml. of concentrated hydrochloric acid is added. The ether solution is separated, washed once with 2 ml. of saturated aqueous sodium sulfate and dried over sodium sulfate. The ether is removed to obtain 3.5 g. of colorless methylmalonic- O_4^{18} acid, m.p. 139°.

(c) *Methyl Methylmalonate- O_4^{18}* . Methylmalonic- O_4^{18} acid, dissolved in ether, is treated with an ether solution of diazomethane. After removal of ether and excess diazomethane methyl methylmalonate- O_4^{18} , b.p. 75° (11 mm.), is obtained by distillation of the residue.

(d) *Methyl 2-Carboxy-3,4-dimethylglutarate-O₄¹⁸*. The "abnormal" Michael condensation of methyl methylmalonate-O₄¹⁸ and ethyl crotonate is carried out according to the conditions described by Michael and Ross,¹ using 0.05 mole quantities and 1 equivalent of sodium ethoxide; see ethyl 2-ethoxycarbonyl-O¹⁸-3,4-dimethylglutarate.

(e) *2-Carboxy-3,4-dimethylglutaric-O₄¹⁸ Acid*. The above methyl 2-carboxy-3,4-dimethylglutarate-O₄¹⁸ is refluxed with 1 N sodium hydroxide to effect hydrolysis. The mixture is cooled in ice, covered with ether and carefully acidified by the dropwise addition of hydrochloric acid, with stirring. The ether phase is separated, dried and then evaporated to dryness. The acid is recrystallized from a mixture of light petroleum, chloroform and ether, m.p. 145°.

B. Notes

1. The methylmalononitrile was prepared from 2-bromopropionic acid by the method of Strack,² which gave low yields.

2. The sodium hydroxide-O¹⁸ solution was prepared from 3% sodium amalgam and water-O¹⁸.

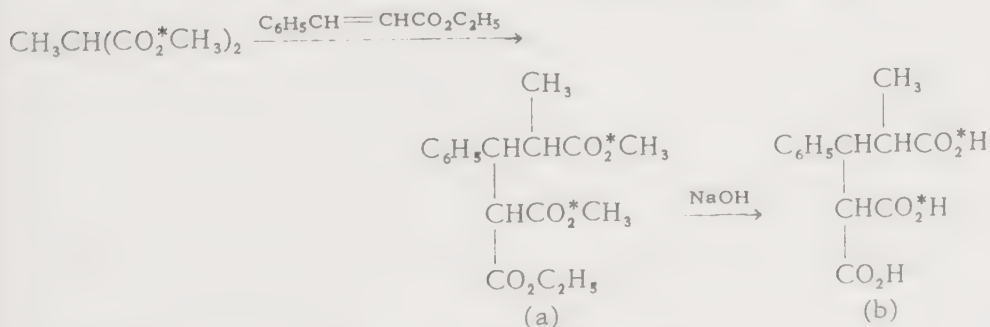
C. Other Preparations

Samuel and Ginsburg also prepared methylmalonic-O₄¹⁸ acid by the hydrolysis of ethyl methylmalonate with sodium hydroxide-O¹⁸ solution.

¹A. Michael and J. Ross, J. Am. Chem. Soc., 52, 4598 (1930).

²E. Strack and H. Schwaneberg, Ber., 67, 41 (1934).

2-CARBOXY-4-METHYL-3-PHENYLGLUTARIC-O₄¹⁸ ACID



D. Samuel and D. Ginsburg, J. Chem. Soc., 1955, 1288.

A. Procedure

(a) *Methyl 2-Carboxy-4-methyl-3-phenylglutarate-O₄¹⁸*. According to the following procedure of Michael and Ross,¹ 20 g. of methyl methyl-

malonate is added to a suspension of sodium ethoxide (prepared from 2.6 g. of sodium) in dry ether. Then 20 g. of ethyl cinnamate is added, and the mixture is refluxed on a water-bath for 6 hours. The mixture is cooled, 7 g. of acetic acid in 50 ml. of water is added, and the ether phase is separated. The aqueous phase is extracted with ether, and the combined ether solution is then washed with 10% sodium carbonate solution and with water. After removal of ether, fractionation of the residual esters gives 16 g. of methyl 2-carbethoxy-4-methyl-3-phenylglutarate, b.p. 185–188° (3 mm.) (Note 1).

(b) *2-Carboxy-4-methyl-3-phenylglutaric-O₄¹⁸ Acid*. Methyl 2-carbethoxy-4-methyl-3-phenylglutarate-O₄¹⁸ is hydrolyzed with 1 *N* sodium hydroxide heated under reflux. The reaction mixture is then chilled in an ice-bath, covered with a layer of ether and carefully acidified by the dropwise addition of hydrochloric acid with stirring. The ether solution is separated and dried. After removal of ether the residual acid is recrystallized from a mixture of light petroleum, chloroform and ether, m.p. 145° (Note 2).

B. Notes

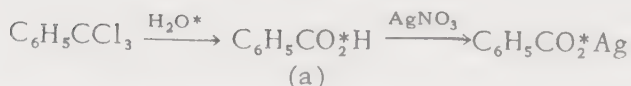
1. For further information regarding the Michael condensation, see ethyl 2-ethoxycarbonyl-O¹⁸-3,4-dimethylglutarate and methyl 2-carbethoxy-3,4-dimethylglutarate-O₄¹⁸.

2. Michael and Ross¹ obtained two apparently stereoisomeric forms of 2-carboxy-4-methyl-3-phenylglutaric acid from the recrystallization. The acid first deposited from solution in a mixture of chloroform, acetone and ligroin melted at 171°. From the mother liquor, a second acid crystallized in fine needles, m.p. 145°.

¹A. Michael and J. Ross, *J. Am. Chem. Soc.*, 52, 4598 (1930).

BENZOIC-O₂¹⁸ ACID

METHOD I



L. Ponticorvo and D. Rittenberg, *J. Am. Chem. Soc.*, 76, 1705 (1954).

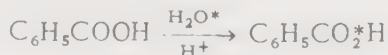
A. Procedure

(a) *Benzoic-O₂¹⁸ Acid*. A mixture of 10 ml. of α, α, α -trichlorotoluene and 20 ml. of water-O¹⁸ is refluxed for 48 hours. After the mixture is chilled, the benzoic-O₂¹⁸ acid is collected and washed with cold water. The wet crystalline mass is dissolved in water with the aid of a minimum of 1 *N*

sodium hydroxide, and the acid is again precipitated by acidification of the solution with mineral acid. After the product is collected, washed with cold water and dried *in vacuo*, the yield is 5.0 g., m.p. 121.6–122° (Note 1).

(b) *Silver Benzoate-O₂¹⁸*. Benzoic-O₂¹⁸ acid is dissolved in 1 N sodium hydroxide. Silver nitrate solution is added, and the precipitate of silver benzoate-O₂¹⁸ is collected, washed with water and dried *in vacuo* at 90°.

METHOD II



I. Roberts and H. C. Urey, J. Am. Chem. Soc., 61, 2580 (1939).

A. Procedure

A solution of 1.7 g. of benzoic acid in 65 ml. of water-O¹⁸, which is 0.1 N in hydrochloric acid, is kept in a bath at 80° for 48 hours. The water and hydrochloric acid are distilled off *in vacuo*, and the residue of benzoic-O₂¹⁸ acid is powdered and dried over phosphorus pentoxide (Notes 2 and 3).

B. Notes

1. It was shown that oxygen-18 in benzoic-O₂¹⁸ acid is completely exchanged with the oxygen of acetic anhydride during 1 hour at the boiling point. The exchange progresses slowly at room temperature.

2. For O¹⁸ analyses a few mg. of the benzoic-O₂¹⁸ acid were decarboxylated in a special glass apparatus over reduced copper.¹ A diagram of the apparatus is shown, and its operation is described by Roberts and Urey. The samples of carbon dioxide-O₂¹⁸ were analyzed in a mass spectrometer. The exchanged benzoic acid contained 0.465% oxygen-18 compared to 0.204% in ordinary benzoic acid. A kinetic study was then made of the exchange reaction at 80° and catalyzed by hydrochloric acid. The rate is first order with respect to the difference of oxygen-18 content of the reactants and independent of the concentration of benzoic acid and free of salt effects.

3. Bender² used essentially the same procedure to prepare benzoic-O₂¹⁸ acid.

C. Other Preparations

In a study³ of the mechanism of oxidation of several aromatic aldehydes with permanganate-O₄¹⁸, the following acids were prepared: benzoic-O₁¹⁸, *m*-chlorobenzoic-O₁¹⁸, *m*-anisic-O₁¹⁸, *p*-anisic-O₁¹⁸, *p*-toluic-O₁¹⁸, *p*-

chlorobenzoic-O¹⁸, *p*-nitrobenzoic-O¹⁸, and piperonylic-O¹⁸. The rates of oxidation were determined from pH 5 to 13. A general acid-catalyzed and a specific hydroxyl ion-catalyzed reaction were observed. In neutral solution, the oxidizing agent appeared to be the source of the oxygen introduced into the aldehyde to form the acid; in basic solution, the solvent contributed a major part of the oxygen.

Potassium permanganate-O¹⁸ has been prepared³ by exchange between potassium permanganate and water-O¹⁸, according to the procedure of Zimmerman.⁴

Hydrocinnamic-O¹⁸ acid has also been prepared⁵ by exchange with water-O¹⁸.

¹P. Sabatier and A. Mailhe, *Compt. rend.*, 159, 217 (1914).

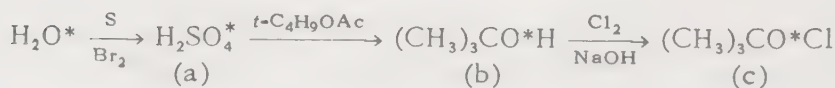
²M. L. Bender, *J. Am. Chem. Soc.*, 73, 1626 (1951).

³K. B. Wiberg and R. Stewart, *J. Am. Chem. Soc.*, 77, 1786 (1955).

⁴G. L. Zimmerman, Thesis, University of Chicago, 1949.

⁵M. L. Bender, R. D. Ginger and K. C. Kemp, *J. Am. Chem. Soc.*, 76, 3350 (1954).

1,1-DIMETHYLETHYL HYPOCHLORITE-O¹⁸ (*t*-Butyl Hypochlorite-O¹⁸)



M. Anbar and I. Dostrovsky, *J. Chem. Soc.*, 1954, 1094.

A. Procedure

(a) *Sulfuric-O¹⁸ Acid*. To 32 g. of pure sulfur, in a 3-necked flask fitted with a reflux condenser and a dropping funnel, is added 80 ml. of water-O¹⁸. To the stirred mixture, 300 g. of bromine is added as fast as it is consumed, as indicated by the disappearance of color. Towards the end of the reaction it is necessary to heat the mixture; when all the sulfur has dissolved, the mixture is distilled until the temperature of the residue reaches 130°. After addition of a few drops of nitric acid (Note 1), the pressure is reduced to 100 mm., and the distillation is continued until the temperature of the residue reaches 220°. The residue is pure sulfuric-O¹⁸ acid (99% concentration).

(b) *2-Methyl-2-propanol-O¹⁸, (t-Butyl Alcohol-O¹⁸)*. To 180 g. of water-O¹⁸ are added 116 g. (1 mole) of *t*-butyl acetate¹ and 10 ml. of sulfuric-O¹⁸ acid (99% concentration). The mixture is refluxed until a clear solution results (about 1 hour). The solution is cooled, metallic calcium equivalent to the sulfuric acid is added, and the solution is filtered. The 2-methyl-2-propanol-O¹⁸, obtained by fractionation of the filtrate, is dried

by azeotropic distillation with benzene; the yield is 70% based on *t*-butyl acetate (Note 2).

(c) *1,1-Dimethylethyl Hypochlorite-O¹⁸*. Chlorine gas is passed through a solution of 20 g. of *t*-butyl alcohol-O¹⁸ in 200 ml. of 10% sodium hydroxide solution. When a layer of *t*-butyl hypochlorite-O¹⁸ has formed, the flow of chlorine is stopped, and the layer of product is separated, washed with 10% sodium carbonate solution and dried over potassium carbonate (Note 3).

B. Notes

1. This is added to oxidize impurities.
2. From the residual solution, left from the isolation of the alcohol, acetic-O₁¹⁸ acid is obtained by distillation.
3. When this preparation was repeated with *t*-butyl alcohol and water-O¹⁸, the isolated *t*-butyl hypochlorite contained no excess O¹⁸.

C. Other Preparations

Anbar and Dostrovsky have prepared *t*-butyl alcohol-O¹⁸ in 30% yield from acetone-O¹⁸, labeled by exchange with water-O¹⁸ and sulfuric acid, and methylmagnesium bromide. They have also prepared *t*-butyl hypochlorite-O¹⁸ from *t*-butyl alcohol-O¹⁸ in an aqueous medium with hypochlorous and perchloric acids.

t-Butyl alcohol-O¹⁸ nitrous ester, bis(1,1-dimethylethanol-O¹⁸) chromate ester and *t*-butyl hypochlorite-O¹⁸ have been prepared² by shaking a mixture of *t*-butyl alcohol-O¹⁸, an aqueous solution of the appropriate acid in dilute sulfuric acid and carbon tetrachloride. *t*-Butyl alcohol-O¹⁸ nitrous ester has also been prepared² from *t*-butyl alcohol-O¹⁸ and liquid nitrogen tetroxide at 0°³ and from *t*-butyl alcohol-O¹⁸ and liquid nitrosyl chloride in pyridine at 0°.

4,4-Dimethyl-3-oxa-2-pentanone-3-O¹⁸ has been prepared² from *t*-butyl alcohol-O¹⁸, acetyl chloride and pyridine in xylene solution, according to the method of Bryant and Smith.⁴

sec-Butyl alcohol-O¹⁸ has been prepared^{5,6} by the reduction of 2-butanone-O¹⁸ with lithium aluminum hydride.

¹Organic Syntheses, Vol. 24, Wiley, New York, 1944, p. 18.

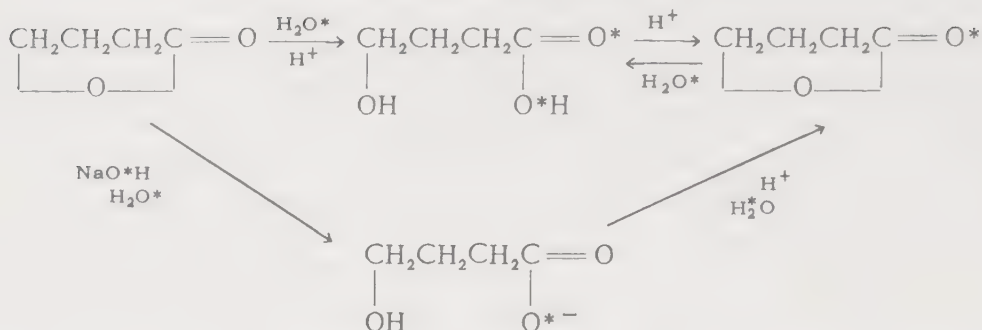
²M. Anbar, I. Dostrovsky, D. Samuel and A. D. Yoffe, J. Chem. Soc., 1954, 3603.

³A. D. Yoffe and P. Gray, J. Chem. Soc., 1951, 1412.

⁴W. M. D. Bryant and D. M. Smith, J. Am. Chem. Soc., 58, 1014 (1936).

⁵I. Dostrovsky and F. S. Klein, J. Chem. Soc., 1955, 4401.

⁶C. A. Bunton, A. Konasiewicz and D. R. Llewellyn, *ibid.*, 1955, 604.

2-OXACYCLOPENTANONE-1-O¹⁸

F. A. Long and L. Friedman, *J. Am. Chem. Soc.*, 72, 3692 (1950); *ibid.*, 75, 2832 (1953).

A. Procedure (Note 1)

(a) 2-Oxacyclopentanone-1-O¹⁸.

(1) *Acid-Catalyzed Hydrolysis in Water-O¹⁸*. To a solution of 0.25 ml. of 96% sulfuric acid in 5 ml. of water-O¹⁸ is added 1 ml. of γ -butyrolactone (0.013 mole). After 100 hours at 23° (Note 2), the O¹⁸₁-lactone (Note 3) is recovered by a continuous extraction of the reaction mixture with benzene for 3 hours. The benzene is removed by distillation, finally under vacuum.

(2) *Base-Catalyzed Hydrolysis in Water-O¹⁸*. To a solution of 5.5 ml. of 3 M sodium hydroxide, made by treating sodium metal with water-O¹⁸, is added 1 ml. of γ -butyrolactone. The hydrolysis reaction, which is very rapid, is permitted to go to completion. Then, 96% sulfuric acid is added in excess of that required to neutralize the base. The resulting acidic solution is set aside for 16 hours at 23° to allow the equilibrium amount of lactone to be reformed. The lactone is then extracted with benzene, as described above (Note 4).

(b) *Other Carbonyl-O¹⁸ Lactones*. The following carbonyl-O¹⁸ labeled lactones were prepared by one or the other of the above procedures: O¹⁸₁- β -propiolactone, O¹⁸₁- γ -valerolactone, O¹⁸₁- γ -crotonolactone, and O¹⁸₁- β -angelicalactone.

B. Notes

1. Two groups of evidence suggest that both the acid- and base-catalyzed hydrolysis of γ -lactones should involve splitting of the acyl-oxygen bond. There is a close similarity of these hydrolysis reactions to those of ordinary aliphatic esters which have been shown to involve acyl-oxygen fission.^{1,2} In addition, both the acid- and base-catalyzed hydrolysis of β -butyrolactone have been shown to involve acyl-oxygen fission, even though the neutral hydrolysis of this lactone does involve

alkyl-oxygen fission.³ Since the kinetics of the hydrolysis of β - and γ -lactones differ considerably in strong acids,⁴ the following experiments were designed to determine the mechanism of both the acid- and the base-catalyzed hydrolysis of γ -butyrolactone.

Finding the location of the isotopic oxygen in the hydrolysis product, by dehydration³ of the resulting O_1^{18} -4-hydroxybutyric acid, was not too successful, and a direct mass spectrometric analysis of the 2-oxacyclopentanone-1- O^{18} was used.

2. This time period was calculated to be several times that required for the reversible reaction to reach equilibrium.

3. The acid-catalyzed hydrolysis of γ -butyrolactone goes to an equilibrium mixture of lactone and 4-hydroxybutyric acid containing about 3 parts of lactone to 1 part of acid. It is known that the oxygens of carboxylic acids exchange with oxygen of the solvent water at a rate comparable with esterification.⁵ Thus, if the hydrolysis of the lactone in water- O^{18} is allowed to go for a time, which is long compared to the time required to reach the hydrolysis equilibrium, both of the carboxyl group oxygens will be equilibrated with the solvent. Since the oxygen of the hydroxyl group does not exchange with the solvent,⁶ the lactone in the equilibrium mixture will contain excess oxygen-18 in the carbonyl group and normal oxygen in the ring.

4. In the basic hydrolysis, assuming the acyl-oxygen fission, the isotopic oxygen in the carboxyl group of the intermediate acid is lost on reforming the lactone in acidic medium. The lactone will contain isotopic oxygen only in the carbonyl as a result of acid-catalyzed exchange with the solvent.

¹M. Polanyi and A. L. Szabo, *Trans. Faraday Soc.*, **30**, 508 (1934).

²S. C. Datta, J. N. Day and C. K. Ingold, *J. Chem. Soc.*, 1939, 838.

³A. R. Olson and J. L. Hyde, *J. Am. Chem. Soc.*, **63**, 2459 (1941).

⁴F. A. Long, F. Dunkle and M. Purchase, unpublished work.

⁵I. Roberts and H. C. Urey, *J. Am. Chem. Soc.*, **61**, 2580 (1939).

⁶O. Reitz, *Z. Elektrochem.*, **45**, 100 (1939).

2-OXAPROPIOPHENONE-2- O^{18}



I. Roberts and H. C. Urey, *J. Am. Chem. Soc.*, **60**, 2391 (1938).

A. Procedure (Note 1)

A reaction mixture is prepared of the following composition: methyl alcohol, 68.6% by volume; hydrochloric acid, 0.123 M; benzoic acid, 2.70

M; water, 2.72 M (4.9% by volume). A 20.4-ml. volume of reaction mixture of the above composition is prepared by dissolving 1 ml. of water and 6.72 g. of benzoic acid in 14.00 ml. of 0.1803 M hydrochloric acid-methanol- O^{18} solution (Note 2). After the reaction period, the water formed and the methanol- O^{18} are distilled from the 2-oxopropiophenone-2- O^{18} , and the water is recovered for isotope analysis by the method of Cohn and Urey.¹ The ester is recovered by vacuum distillation (Note 3).

B. Notes

1. This is a mechanism study and therefore does not represent ideal conditions of esterification from a preparative point of view. Attempts to make the reaction go to completion in a short time resulted in esterification of the large amount of hydrochloric acid catalyst which must be used. Therefore, the concentrations of catalyst and reactants were so adjusted as to give a half-time of about two weeks at 25° . Several procedures for the preparation of methyl benzoate are given in the literature.²⁻⁴ The latter reference reports a 95% yield of this ester.

2. The rate of esterification at 24.93° was determined using a solution of this composition but containing ordinary methanol. Samples removed at intervals were titrated with standard sodium hydroxide solution to determine the benzoic acid remaining. The reaction was 55.17% complete after 383.0 hours.

3. The water removed after 94.0, 212.4 and 334.0 hours of reaction did not contain excess oxygen-18; hence the oxygen in the water formed in the esterification originates entirely in the benzoic acid.

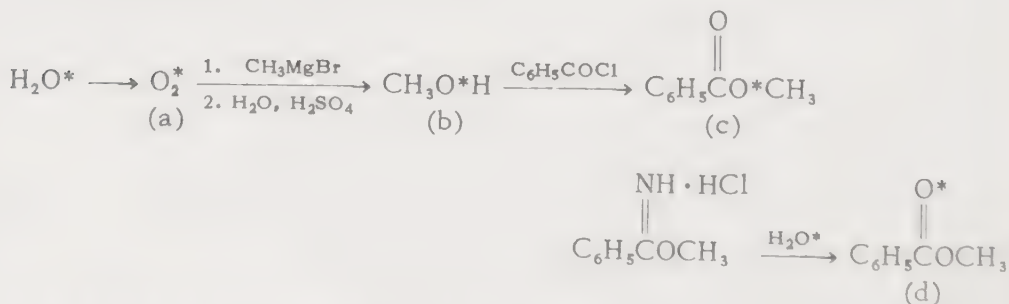
¹M. Cohn and H. C. Urey, *J. Am. Chem. Soc.*, **60**, 679 (1938).

²F. J. Sowa and J. A. Nieuwland, *ibid.*, **58**, 271 (1936).

³F. Rinderknecht and C. Niemann, *ibid.*, **70**, 2605 (1948).

⁴R. O. Clinton and S. C. Laskowski, *ibid.*, **70**, 3135 (1948).

2-OXAPROPIOPHENONE-2- O^{18} AND 2-OXAPROPIOPHENONE-1- O^{18}



K. B. Wiberg, *J. Am. Chem. Soc.*, **75**, 2665 (1953).

A. Procedure

(a) *Oxygen-O¹⁸*. The enriched oxygen is obtained by the electrolysis of a 20% solution of sulfuric acid in water-O¹⁸. The electrolysis cell is a U-tube constructed from a 33-cm. piece of 14-mm. tubing. The platinum electrodes are sealed in. Two bulbs of 30-ml. capacity are attached to the hydrogen side such that the electrolyte will flow into the first bulb when the flow of oxygen is restricted during the reaction, thus stopping the electrolysis. The second bulb prevents water, which is used as a controllable head, from entering the electrolysis cell. A tube leading to the bottom of the cell is used to stir the electrolyte occasionally with nitrogen. The cell is immersed in cold water during the electrolysis and is swept out with nitrogen before use. The oxygen-O₂¹⁸ is passed through a Dry Ice-acetone cooled trap to remove water vapor. A current of 4 amperes passed through the cell for 7 hours generates about 0.5 g. atom of oxygen.

(b) *Methanol-O¹⁸*. About 0.25 mole of oxygen-O₂¹⁸ is introduced into 0.55 mole of methylmagnesium bromide in 500 ml. of ether, under a nitrogen atmosphere, in a one-liter 3-necked flask having a stirrer and a reflux condenser (Note 1). After the oxygen is introduced, the excess ether is decanted from the precipitated salt; ice, followed by 25% sulfuric acid, is added with external cooling. The solution thus obtained is distilled through an 18-cm. column packed with "Helipak"; two fractions are collected: boiling range 60-70° and boiling range 70-99°. The higher-boiling fractions from several runs are redistilled, and the material boiling from 60-70° is collected. The lower-boiling fractions are then combined and redistilled to obtain 50-70% of methanol-O¹⁸, b.p. 65°.

(c) *2-Oxapropiophenone-2-O¹⁸*. To 8 g. (0.1 mole) of pyridine is added 3.2 g. (0.1 mole) of methanol-O¹⁸ and then 14 g. (0.1 mole) of benzoyl chloride, with ice-bath cooling. After water is added, the oily layer which separates is washed with water and distilled. The yield of ether-labeled methyl benzoate, b.p. 80° (15 mm.), is 10 g. (77%) (Note 2).

(d) *2-Oxapropiophenone-1-O¹⁸* (Note 3). This compound is prepared by the hydrolysis of methyl benzimidate hydrochloride according to the procedure of Bender;¹ see 2-oxabutyrophenone-1-O¹⁸.

B. Notes

1. The delivery tube is of large diameter to prevent clogging by the precipitated salt.

2. The product had essentially no oxygen-O¹⁸ in the carbonyl group as determined from the mass spectrum.

3. The labeled methyl benzoates were used in a thermal rearrangement study. About 66% rearrangement occurred at 400-405° during 3 hours.

This rearrangement is similar to that of vinyl ethers² and benzimidic ethers.³

C. Other Preparations

Methanol- O^{18} has been obtained by the fractional distillation of ordinary methanol;⁴ and by the hydrolysis⁵ of methyl phosphate in acid solution containing water- O^{18} . The former of these methods is inconvenient and leads to only a small degree of enrichment under practical conditions. In the latter method, considerable methyl ether forms during the reaction, and a product having much less oxygen-18 than the water- O^{18} used is obtained. In addition, the conversion of water- O^{18} to methanol is not favorable unless the water is repeatedly recycled.

¹M. L. Bender, J. Am. Chem. Soc., 73, 1626 (1951).

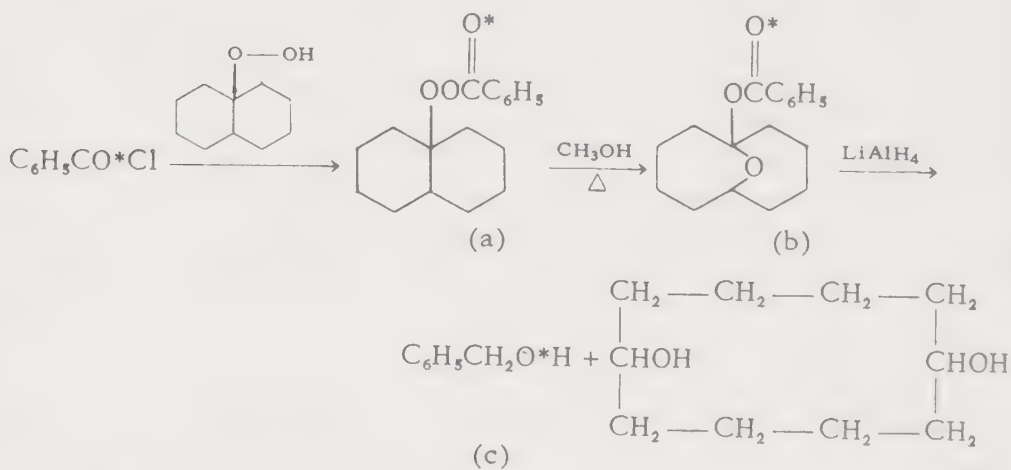
²L. Claisen, Ber., 29, 2931 (1896).

³A. W. Chapman, J. Chem. Soc., 1927 (1743).

⁴I. Roberts and H. C. Urey, J. Am. Chem. Soc., 60, 2391 (1938).

⁵E. Blumenthal and J. B. M. Herbert, Trans. Faraday Soc., 41, 611 (1945).

2-OXA-2-(1,6-EPOXYCYCLODECYL)ACETOPHENONE-1- O^{18} (1-Benzoyl- O^{18} -oxy-1,6-epoxycyclodecane)



D. B. Denney, J. Am. Chem. Soc., 77, 1706 (1955).

A. Procedure (Note 1)

(a) *trans*-4a-(Benzoyl- O^{18} -peroxy)decahydronaphthalene. According to the following procedure of Cope and Holzman,¹ 9.95 g. of 4a-decahydronaphthyl hydroperoxide is dissolved in 25 ml. of pyridine. To this solution, cooled below 10° , is added 9.85 g. of benzoyl chloride, with stirring, during 5 minutes. After the solution is kept at 25° for 1 hour, it is poured into 200 ml. of cold 10% sulfuric acid. The crystalline product is

collected and washed with two 20-ml. portions of methanol. The yield of the ester, m.p. 61–65°, is 14.75 g. (92%). After the product is dissolved in methanol at room temperature and recrystallized by cooling the solution, the m.p. is 67–68° (Note 2).

(b) 2-Oxa-2-(1,6-epoxycyclodecyl)acetophenone-1-O¹⁸, (1-Benzoyl-O¹⁸-oxy-1,6-epoxycyclodecane). *trans*-4a-(Benzoyl-O¹⁸-peroxy)decahydronaphthalene is rearranged in either methanol or acetic acid (Note 3). The ester, 5 g., is heated under reflux with 10 ml. of methanol for 1 hour on a steam-bath.¹ When the solution is cooled, 3.5 g. (70%) of 2-oxa-2-(1,6-epoxycyclodecyl)acetophenone-1-O¹⁸, m.p. 93–95°, separates; 0.3 g. (6%), m.p. 80–90°, is isolated by concentration of the filtrate.

(c) *Benzyl Alcohol-O¹⁸*. Reduction of the above compound (b) with lithium aluminum hydride gives benzyl alcohol-O¹⁸ and 1,6-cyclodecanediol.

B. Notes

1. Experimental details were not given.
2. This m.p. agrees with that reported by Criegee.²
3. Criegee and Kaspar³ have shown that the ester undergoes rearrangement rapidly in solvents of high ionizing power and slowly in poorly ionizing solvents. They have also shown that the rearrangement involves an ionic fission of the oxygen-oxygen bond and takes a course analogous to the Wagner-Meerwein rearrangement in which there is an ionic fission of a carbon-halogen or carbon-oxygen link. Bartlett and Kice⁴ have made a kinetic study of the rearrangement.

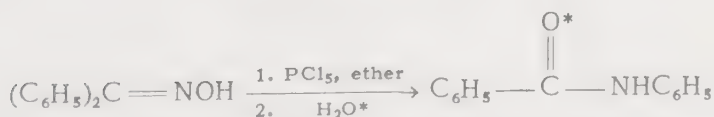
¹A. C. Cope and G. Holzman, J. Am. Chem. Soc., 72, 3062 (1950).

²R. Criegee, Ber., 77B, 22 (1944).

³R. Criegee and R. Kaspar, Ann., 560, 127 (1948).

⁴P. D. Bartlett and J. L. Kice, J. Am. Chem. Soc., 75, 5591 (1953).

BENZANILIDE-O¹⁸



A. E. Brodsky and G. P. Michluchin, Comp. rend. acad. sci. U.R.S.S. 32, 558 (1941); Chem. Abstracts, 37, 1710 (1943); *ibid.*, 43, 5011 (1949).

A. Procedure (Note 1)

To a solution of 2 g. of benzophenone oxime in 20 ml. of anhydrous ether at –15° (Note 2) is added 3 g. of powdered phosphorus pentachloride (Note 3). The solvent is removed on a water bath and 25 ml. of water-O¹⁸ is added to the residue. The water is refluxed for a few min-

utes and any lumps which form are broken up. The product is collected and recrystallized from alcohol, m.p. 163° (Note 4).

B. Notes

1. This is a study of the Beckmann rearrangement of ketoximes, using oxygen- O^{18} . Stieglitz¹ has proposed a mechanism in which the dehydration, rearrangement and hydrolysis of an ammonium salt-like intermediate occurs, as adverse to a direct rearrangement without dehydration as an intermediate stage. Barring an exchange between water- O^{18} and benzanilide, the appearance of excess isotope in the benzanilide formed in the rearrangement is evidence in favor of the mechanism of Stieglitz. Michluchin and Brodsky² have shown that benzanilide does not exchange oxygen with water- O^{18} , nor does it exchange under the conditions of the rearrangement with phosphorus pentachloride present.

2. The rearrangement, as done by Brodsky, was performed at a low temperature to suppress the reaction of benzanilide with phosphorus pentachloride.³

3. This procedure is in accordance with that described by Vogel.⁴

4. Hydrogenation of the benzanilide- O^{18} at 90 atmospheres in the presence of a molybdenum sulfide catalyst, followed by careful purification and assay of the water- O^{18} formed, indicated that the rearrangement is not intramolecular but probably follows the mechanism of Stieglitz.¹

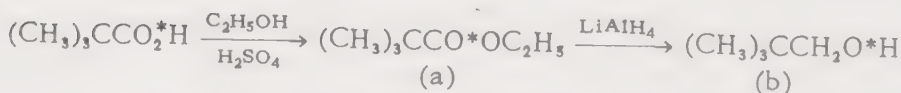
¹J. Stieglitz and P. N. Leech, J. Am. Chem. Soc., 36, 272 (1914).

²G. P. Michluchin and A. E. Brodsky, Acta Physicochim. U.R.S.S., 16, 63 (1942); Chem. Abstracts, 37, 2355 (1943).

³V. O. Wallach and M. Hoffmann, Ann., 184, 79 (1877).

⁴A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., New York, 1948, p. 705.

2,2-DIMETHYL-1-PROPANOL- O^{18}



I. Dostrovsky and F. S. Klein, J. Chem. Soc., 1955, 4401.

A. Procedure

(a) *2,2-Dimethyl-4-oxa-3-hexanone-3- O^{18}* . 2,2-Dimethylpropionic- O^{18} acid (pivalic- O^{18} acid) is esterified with ethanol and sulfuric acid catalyst (Note 1).

(b) *2,2-Dimethyl-1-propanol- O^{18}* . A solution of 18 g. of 2,2-dimethyl-4-oxa-2-hexanone-3- O^{18} in 60 ml. of dry ether, is added dropwise to a suspension of 30 g. of lithium aluminum hydride in 70 ml. of ether. After the excess of hydride is decomposed with a water-alcohol-ether mixture, the

reaction mixture is poured into a solution of sulfuric acid containing ice. The 2,2-dimethyl-1-propanol- O^{18} is separated and fractionated through a 20-plate column; yield, 60%, m.p. 54.9° .

B. Notes

1. A general method for the esterification of a number of organic acids was described by Brändström,¹ using sulfuric acid as catalyst and azeotropic removal of water with a Widequist water-separator² (Figure XVIII, 1). The yield of ethyl pivalate (ethyl 2,2-dimethylpropionate), b.p. $117-118^{\circ}$, was 91%.

¹A. Brändström, *Arkiv Kemi*, 1, 481 (1950).

²S. Widequist, *Acta Chem. Scand.*, 3, 303 (1949); *Chem. Abstracts*, 43, 7901 (1949).

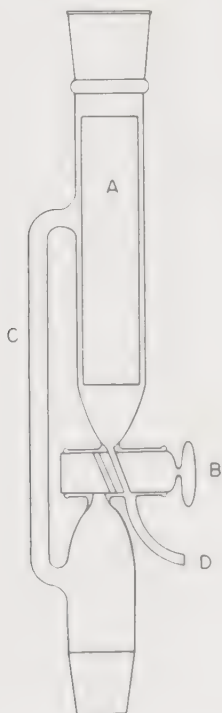
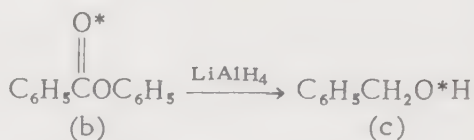
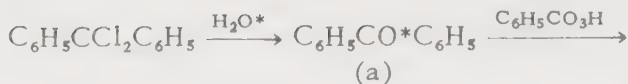


Fig. XVIII, 1 Widequist water separator for continuous azeotropic removal of water with solvents of density greater than 1 (S. Widequist). A, inner open glass tube; B, two-way stopcock; C, solvent return tube; D, drain for water removal.

BENZYL ALCOHOL-O¹⁸

W. von E. Doering and E. Dorfman, J. Am. Chem. Soc., 75, 5595 (1953).

A. Procedure

(a) *Benzophenone-O¹⁸*, (*Diphenyl Ketone-O¹⁸*). A mixture of 35 g. of dichlorodiphenylmethane and 9.7 g. of water-O¹⁸ is stirred at 100° for 3 hours in a 2-necked flask equipped with mercury-sealed stirrer, reflux condenser and calcium chloride drying tube. The resulting product is collected and recrystallized once from isoöctane and twice from hexane. The yield of benzophenone-O¹⁸, m.p. 47–48°, is 24.3 g.

(b) *2-Oxa-2-phenylacetophenone-2-O¹⁸* (Note 1). To a solution of 35 g. of perbenzoic acid in 1100 ml. of benzene is added 20.0 g. of benzophenone-O¹⁸, and the resulting solution is kept in the dark for 10 days. The benzene solution is washed once with aqueous sodium carbonate, three times with water and then concentrated. The residue is fractionally crystallized from isoöctane to obtain 1.0 g. of benzophenone-O¹⁸, m.p. 40–44°, and 18.0 g. of 2-oxa-2-phenylacetophenone-2-O¹⁸, m.p. 61–66°. After recrystallization from ethanol, the 2-oxa-2-phenylacetophenone-2-O¹⁸ melts at 68–69.5°.

(c) *Benzyl Alcohol-O¹⁸* (Note 2). To 600 ml. of anhydrous ether in a one-liter 3-necked flask, equipped with mercury-sealed stirrer, reflux condenser, drying tube and dropping funnel, 4.0 g. of lithium aluminum hydride is added. After the mixture is stirred for 30 minutes, 7.0 g. of 2-oxa-2-phenylacetophenone-2-O¹⁸ is added over a period of 10 minutes. The mixture is stirred for 5 hours more and set aside for an additional 12 hours; then water is added cautiously. The precipitate is filtered off and washed with ether. The combined ether solution is washed with 700 ml. of 5% sodium hydroxide solution in 100-ml. portions and then with 300 ml. of water in 100-ml. portions (Note 3). The ether solution, dried over magnesium sulfate, is concentrated and then distilled. The yield of benzyl alcohol-O¹⁸, b.p. 49° (1 mm.), is 3.0 g. By evaporative distillation at 5 mm., product with n_D^{25} 1.5372 is obtained.

B. Notes

1. The oxidation of benzophenone-O¹⁸ with perbenzoic acid served to decide the mechanism in the peracid ketone-ester conversion. Of the

three mechanisms under consideration: the "Criegee" mechanism^{1,3} would lead to carbonyl labeled phenylbenzoate, the "Wittig" mechanism² would give ether-labeled phenyl benzoate, and the "v. Baeyer" mechanism^{2,4} would result in an ester with the oxygen isotope equally distributed between the two oxygens of the ester.

2. What appears to be a very excellent method of analyzing organic molecules for excess oxygen isotope was developed. The oxygen-18 was obtained as carbon dioxide-O₂¹⁸ and analyzed directly in the mass spectrograph.

3. The final washings did not contain phenol.

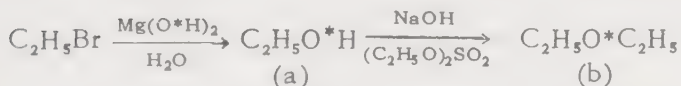
¹R. Criegee, *Ann.*, 560, 127 (1948).

²W. von E. Doering and L. Speers, *J. Am. Chem. Soc.*, 72, 5515 (1950).

³S. L. Friess, *ibid.*, 71, 2571 (1949).

⁴A. v. Baeyer and V. Villiger, *Ber.*, 32, 3625 (1899).

ETHYL ETHER-O¹⁸



I. Lauder and J. H. Green, *Nature*, 157, 767 (1946); *Trans. Faraday Soc.*, 44, 808 (1948).

A. Procedure

(a) *Ethanol-O¹⁸*, (*Ethyl Alcohol-O¹⁸*). Magnesium oxide, 1 g., and 4 g. of water-O¹⁸ are heated in a heavy-walled, sealed Pyrex tube for 24 hours to establish isotopic equilibrium. To this mixture, 4.5 g. of ethyl bromide is added, and the tube is cooled, evacuated, and resealed (Note 1). With constant shaking, the mixture is heated in a bath at 110° for 3-7 days. The tube is cooled and opened, and the volatile products are pumped off while the temperature of the residue is gradually raised to 100°. The aqueous alcohol, containing a trace of ethyl bromide, is transferred to a small distilling apparatus. On warming the solution any trace of ethyl bromide is removed, and the fraction distilling up to 100° is then collected. The distillate is redistilled until the product reaches the desired ratio of water to ethanol (Note 2).

(b) *Ethyl Ether-O¹⁸* (Note 3). A flask containing a mixture of 11.7 mmoles of ethyl sulfate, 8.95 mmoles of sodium hydroxide, 8.95 mmoles of ethanol-O¹⁸ and 9.8 mmoles of water-O¹⁸ is cooled, evacuated and closed. The flask and contents are then shaken at room temperature (Note 4). In the presence of base the rapid, exothermic reaction may require cooling. Ether-O¹⁸ is recovered by cooling the reaction mixture to -60° and distillation of the ether under vacuum.

B. Notes

1. Ethyl bromide and ethyl alcohol form an azeotrope, b.p. 37.6° , containing 7 mole per cent alcohol. Therefore, it was desirable to have slightly more magnesium hydroxide present than was required for the hydrolysis of all the ethyl bromide, to avoid loss of the product during the subsequent distillation.

2. For the etherification reaction, an approximately 1:1 mixture of alcohol and water was desired. The composition of the distillate was determined with a small hydrometer.

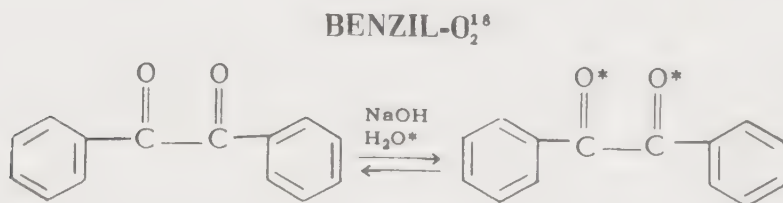
3. The reaction of ethyl sulfate with ethanol occurs readily at room temperature in the presence of an equivalent of alkali and a small amount of water.¹

4. The excess oxygen-18 content of ether, withdrawn at intervals, was determined by converting the oxygen to water² and measuring its excess density.³

¹A. R. Cade, Chem. & Met. Eng., 29, 319 (1923).

²I. Lauder and J. B. M. Herbert, Trans. Faraday Soc., 34, 432 (1938).

³E. S. Gilfillan, Jr. and M. Polanyi, Z. physik. Chem., 166, 254 (1933).



I. Roberts and H. C. Urey, J. Am. Chem. Soc., 60, 880 (1938).

A. Procedure

A mixture of 8.75 g. (0.0833 equivalent, 0.0417 mole) of benzil and 20 ml. of methanol are heated to boiling (Note 1). For experiments with basic catalysis, 0.030 ml. of 50% sodium hydroxide is also added initially. Into the resulting solution is pipetted 1.5 ml. (0.0833 mole) of water-O¹⁸. The solution is again heated to the boiling point until complete solution is obtained and immediately chilled under the tap, causing most of the dissolved solid to precipitate (Note 2). To recover the water for isotope analysis, all liquid is distilled from the mixture *in vacuo* at room temperature and then fractionated. In the base catalyzed reactions, exchange is complete in 4 minutes and 43% complete in the same time without added base.

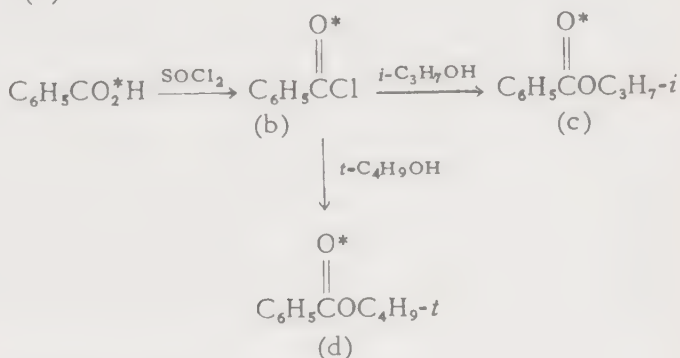
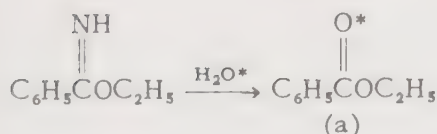
B. Notes

1. Since benzil is only slightly soluble in water, methanol was added as a mutual solvent. Experiments indicated that exchange of oxygen be-

tween methanol and water, under the conditions employed, was not measurable.

2. Under these conditions of oxygen exchange, rearrangement of benzil to benzoic acid took place to a negligible degree; therefore the product was practically all benzil- O_2^{18} .

2-OXABUTYROPHENONE-1- O^{18}



M. L. Bender, J. Am. Chem. Soc., 73, 1626 (1951).

A. Procedure (Note 1)

(a) *2-Oxabutyrophenone-1- O^{18}* . Ethyl benzimidate hydrochloride¹ (46 g., 0.248 mole) is dissolved in a mixture of 46.2 ml. of doubly distilled water and 3.8 ml. of water containing about 1.5 atom per cent O^{18} . The solution is heated on a steam-bath for 1 hour with occasional shaking. The resulting mixture is cooled; the organic layer is separated, washed with water, dried with sodium sulfate and distilled through a column packed with glass helices. The yield of 2-oxabutyrophenone-1- O^{18} , b.p. 90° (14 mm.), is 30 g. (81%); n_D^{20} 1.5053.

(b) *Benzoyl- O^{18} Chloride*. Benzoic- O_2^{18} acid (22 g., 0.18 mole) is mixed with 27 g. (0.226 mole) of thionyl chloride. The mixture is kept at room temperature for 2 hours and then refluxed for 2 hours. Excess thionyl chloride and hydrogen chloride are removed *in vacuo* leaving a residue of benzoyl- O^{18} chloride.

(c) *3-Methyl-2-oxabutyrophenone-1- O^{18}* . A mixture of benzoyl- O^{18} chloride (0.18 mole) and 20 g. of isopropyl alcohol is refluxed for 12 hours. The reaction product is washed with 5% potassium carbonate solution and then with water, dried over sodium sulfate and distilled through a column. The yield of ester is 25 g. (85%); b.p. $71\text{--}72^\circ$ (5 mm.); n_D^{20} 1.4949.

(d) 3,3-Dimethyl-2-oxabutyrophenone-1-O¹⁸ (Note 2). A mixture of 0.18 mole of benzoyl-O¹⁸ chloride, 24.7 ml. of *t*-butyl alcohol and 31.3 ml. of pyridine is set aside overnight. The reaction mixture is extracted twice with water, four times with 0.1 *N* hydrochloric acid and finally twice more with water. The crude ester then is dried over sodium sulfate and distilled through a column. The yield of ester is 22 g. (66%); b.p. 67–68° (1 mm.); n_D^{25} 1.4890.

B. Notes

1. For purposes of O¹⁸ analysis, carbon dioxide was obtained from the esters in two steps in essentially quantitative yield. The first step was the thermal decomposition of the ester into benzoic acid and an olefin, according to Bilger.² The second step, decarboxylation of benzoic acid, was as described by Roberts.³

Rates of alkaline hydrolysis of the labeled esters were determined in water and in 33% dioxane-water. The rate of acid hydrolysis of ethyl benzoate was also determined. In every case exchange of oxygen took place between the solvent and the ester during the hydrolysis. The author presents a theory of hydrolysis mechanism on the basis of the results.

2. The preparation of 3,3-dimethyl-2-oxabutyrophenone-1-O¹⁸ was according to the procedure of Norris.⁴

C. Other Preparations

Benzoyl-O¹⁸ chloride, prepared^{5,6} essentially by the method described, has been converted^{5,6} to benzamide-O¹⁸, m.p. 127.5° after recrystallization from water, in 90% yield by reaction with anhydrous ammonia.

Hydrocinnamoyl-O¹⁸ chloride and 2-oxa-5-phenyl-3-pentanone-3-O¹⁸ have been prepared from hydrocinnamic-O₂¹⁸ acid by procedures similar to those described.⁶

¹A. Pinner, *Ber.*, 16, 1654 (1883).

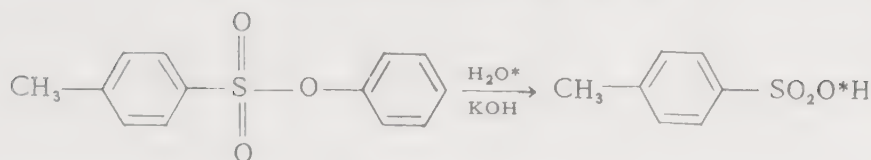
²E. M. Bilger and H. Hibbert, *J. Am. Chem. Soc.*, 58, 823 (1936).

³I. Roberts and H. C. Urey, *ibid.*, 61, 2580 (1939).

⁴J. F. Norris and G. W. Rigby, *ibid.*, 54, 2088 (1932).

⁵M. L. Bender and R. D. Ginger, *ibid.*, 77, 348 (1955).

⁶M. L. Bender, R. D. Ginger and K. C. Kemp, *ibid.*, 76, 3350 (1954).

p-TOLUENESULFONIC-O¹⁸ ACID

C. A. Bunton and Y. F. Frei, J. Chem. Soc., 1951, 1872.

A. Procedure (Note 1)

The hydrolysis experiments are carried out in aqueous dioxane (4:6 V/V), to which are added the required amounts of solid potassium hydroxide; a correction is applied for the dilution of isotopic oxygen in the water by the normal oxygen of the potassium hydroxide. The experimental conditions and results are given in the following table.

TABLE XVIII, 1

Hydrolysis of Benzene *p*-Toluenesulfonate

Ml. of solvent	Moles of ester	Moles of KOH	Time, days	Temp., °C.	Atom % excess O ¹⁸	
					initial	phenol
180	0.016	0.025	20	100	0.528	0.006
200	0.02	0.25	8	100	0.517	0.011
130	0.02	0.25	6	120	0.502	0.013
200	0.02	40	120	No reaction	

After the solution is treated with a stream of carbon dioxide, the liberated phenol is distilled with steam, isolated and dried over phosphorus pentoxide (Note 2). After acidification and evaporation of the residual solution, the *p*-toluenesulfonic-O¹⁸ acid is isolated by extraction with absolute alcohol and/or sublimation (Note 3).

B. Notes

1. It has been shown, using water with excess oxygen-O¹⁸, that the hydrolysis of alkyl sulfonates results in alkyl-oxygen bond fission.¹ The reduction of alkyl sulfonates by lithium aluminum hydride involves alkyl-oxygen bond fission, with formation of a hydrocarbon; however, aryl sulfonates are reduced by this reagent to phenols, involving fission of the sulfur-oxygen bond.^{2,3} It was expected that this alternative mode of nucleophilic substitution, involving attack on the sulfur atom, should appear in the hydrolysis of compounds whose structure restricts attack on the carbon atom involved in the oxygen-carbon bond. Nucleophilic attack on a phenyl group is usually difficult, as in the hydrolysis of the

halogeno-benzenes. The hydrolysis of phenyl *p*-toluenesulfonate illustrates the hydrolysis of an unsubstituted phenyl sulfonate involving nucleophilic attack on the sulfur atom, with breaking of the sulfur-oxygen bond.

2. The oxygen of the phenol is converted to water by the method of Russell,⁴ which is then purified and equilibrated with carbon dioxide by the method of Cohn,⁵ followed by mass spectrometric analysis of the latter.

3. *p*-Toluenesulfonic acid, which is soluble in alcohol and slightly soluble in water, melts at 104-5° and boils at 146-7° (25 mm.).

¹Ader, Thesis, London, 1949.

²H. Schmid and P. Karrer, *Helv. Chim. Acta*, 32, 1371 (1949).

³G. W. Kenner and M. A. Murray, *J. Chem. Soc.*, 1950, 406.

⁴W. W. Russell and J. W. Fulton, *Ind. Eng. Chem., Anal. Ed.*, 5, 384 (1933).

⁵M. Cohn and H. C. Urey, *J. Am. Chem. Soc.*, 60, 679 (1938).

TABLE XVIII, 2

Hydrolysis of Esters with Water-O¹⁸

Ester		Water-O ¹⁸		Catalyst	Temp., °C.	Time	Isotopic Product	Notes	Ref.
Formula	Name	Amount	mmoles						
C ₂ H ₃ Ag ₂ O ₅ P	Disilver acetyl phosphate	150 mg.	1 ml. 95 mg. KOH	18 hrs.	Acetic-O ¹⁸ acid	Alkaline hydrolysis fissions the oxygen-carbon bond.	1
C ₂ H ₃ Ag ₂ O ₅ P	Disilver acetyl phosphate	150 mg.	1 ml. 35 mg. NaCl	85	2 hrs.	Phosphoric-O ¹⁸ acid	The solution in this case was acidic. Acid hydroly- sis fissions the oxygen- phosphorus bond.	1
C ₃ H ₉ O ₄ P	Trimethyl phosphate	2	14 mmoles	100- 110	18-24 hrs.	Methanol-O ¹⁸	The methyl-oxygen bond is broken; the pH decreases during reaction.	2
C ₃ H ₉ O ₄ P	Trimethyl phosphate	2	14 mmoles	100- 110	18-24 hrs.	Methanol-O ¹⁸	The methyl-oxygen bond is broken.	2
C ₃ H ₉ O ₄ P	Trimethyl phosphate	2	14 mmoles	100- 110	18-24 hrs.	Dimethyl hydrogen phosphate- O ¹⁸	The phosphorus-oxygen bond is broken.	2
C ₄ H ₉ BrO	<i>t</i> -Butyl hypo- bromite NaOH	NaO*Br	No alkyl-oxygen bond fission.	6
C ₄ H ₉ ClO	<i>t</i> -Butyl hypo- chlorite NaOH	NaO*Cl	No alkyl-oxygen bond fission.	6
C ₄ H ₉ NO ₂	Butyl nitrite NaOH	NaNO*	No alkyl-oxygen bond fission.	6
C ₄ H ₉ NO ₂	<i>t</i> -Butyl nitrite NaOH	NaNO*	No alkyl-oxygen bond fission.	6
C ₄ H ₉ NO ₃	Butyl nitrate NaOH	NaNO* and Butanol-O ¹⁸	Alkyl-oxygen bond fission, 33%.	6

(Continued)

TABLE XVIII, 2 (Continued)

Ester			Water-O ¹⁸	Catalyst	Temp., °C.	Time	Isotopic Product	Notes	Ref.
Formula	Name	Amount mmoles							
C ₄ H ₉ NO	<i>t</i> -Butyl nitrate	NaOH	<i>t</i> -Butyl alcohol-O ¹⁸	Alkyl-oxygen bond fission, 100%.	6
C ₄ H ₉ NaO ₄ S	Sodium butyl sulfate	38 g.	30 ml.	10 N NaOH	b.p.	2 days	Butanol-O ¹⁸	Alkyl-oxygen bond fission.	7
C ₄ H ₁₀ O ₄ S	Ethyl Sulfate	NaOH	Na ₂ SO ₄ * and Ethanol-O ¹⁸	Alkyl-oxygen bond fission, 85%.	6
C ₃ H ₈ O ₄	Methyl hydrogen succinate	13.05 g.	22.18 g. of solution	0.3044 N HCl	100	24 hrs.	Succinic-O ¹⁸ acid	In acid catalyzed hydrolysis of esters the carbonyl-oxygen bond is broken.	3
C ₇ H ₁₄ O ₂	Amyl acetate	1.8	NaO ¹⁸ H	70	48 hrs.	Acetic-O ¹⁸ acid	In base catalyzed hydrolysis of esters the carbonyl-oxygen bond is broken.	4
C ₈ H ₁₂ O ₈ Si	Tetracetoxysilane	Acetic-O ¹⁸ acid	The carbonyl oxygen bond is broken.	5
C ₆ H ₁₂ O ₂	<i>t</i> -Butyl acetate	NaOH	Acetic-O ¹⁸ acid	Alkyl-oxygen bond fission, 4%.	6
C ₈ H ₁₇ NO ₃	Octyl nitrate	NaOH	NaNO ₃ * and Octyl-O ¹⁸ alcohol	Alkyl-oxygen bond fission, 15%.	6
C ₈ H ₁₈ CrO ₄	<i>t</i> -Butyl chromate	NaOH	Na ₂ CrO ₄ *	Alkyl-oxygen bond fission, 4%.	6
C ₈ H ₂₀ O ₄ Si	Ethyl silicate	None	78	Silicic-O ¹⁸ acid	The oxygen-silicon bonds are broken in neutral, acidic and basic media.	5
C ₁₅ H ₁₇ O ₅ P	Acetyl dibenzyl phosphate	150 mg.	1 ml.	140 mg. KOH	72 hrs.	Acetic-O ¹⁸ acid	Alkaline hydrolysis breaks the oxygen-carbon bond.	1

(Continued)

$C_{10}H_{15}BrO_3$	Triphenyl- methyl bromate	NaOH	NaBrO ₃ *	No alkyl-oxygen bond fission.	6
$C_{10}H_{15}ClO$	Triphenyl- methyl hy- pochlorite	NaOH	NaO*Cl	No alkyl-oxygen bond fission.	6
$C_{10}H_{15}ClO_3$	Triphenyl- methyl chlorate	NaOH	NaClO ₃ *	Alkyl-oxygen bond fission, 1%.	6
$C_{10}H_{15}ClO_4$	Triphenyl- methyl perchlorate	NaOH	NaClO ₄ * and triphenyl- methanol-O ¹⁸	Alkyl-oxygen bond fission, 95%.	6
$C_{10}H_{15}IO_3$	Triphenyl- methyl iodate	NaOH	NaIO ₃ *	Alkyl-oxygen bond fission, 1%.	6
$C_{10}H_{15}NO_2$	Triphenyl- methyl nitrite	NaOH	NaNO ₂ *	Alkyl-oxygen bond fission, 1%.	6
$C_{10}H_{15}NO_3$	Triphenyl- methyl nitrate	NaOH	NaNO ₃ * and triphenyl- methanol-O ¹⁸	Alkyl-oxygen bond fission, 91%.	6
$C_{21}H_{18}O_2$	Triphenyl- methyl acetate	NaOH	Triphenyl- methanol-O ¹⁸ and acetic- O ¹⁸ acid	Alkyl-oxygen bond fission, 67%.	6
$C_{21}H_{18}O_3$	Triphenyl- methyl acetate	80% dioxane	initially neutral	Triphenyl- methanol-O ¹⁸ and acetic- O ¹⁸ acid	Alkyl-oxygen bond fission, 95%.	8

TABLE XVIII, 2 (Continued)

Ester			Water-O ¹⁸			Temp., °C.	Time	Isotopic Product	Notes	Ref.
Formula	Name	Amount mmoles		Catalyst						
C ₂₁ H ₁₈ O ₂	Triphenyl- methyl acetate	80%	0.015 M dioxane NaOH	Triphenyl- methanol-O ¹⁸ and acetic- O ¹⁸ acid	Alkyl-oxygen bond fission, 78%.	8
C ₂₁ H ₁₈ O ₂	Triphenyl- methyl acetate	80%	0.028 M dioxane LiOH	Triphenyl- methanol-O ¹⁸ and acetic- O ¹⁸ acid	Alkyl-oxygen bond fission, 83.5%.	8
C ₂₁ H ₁₈ O ₂	Triphenyl- methyl acetate	80%	0.0446 M dioxane HClO ₄	Triphenyl- methanol-O ¹⁸ and acetic- O ¹⁸ acid	Alkyl-oxygen bond fission, 94%.	8
C ₃₈ H ₃₀ CrO ₄	Triphenyl- methyl chromate	NaOH	Triphenyl- methanol-O ¹⁸ and Na ₂ CrO ₄ [*]	Alkyl-oxygen bond fission, 66%.	6
C ₃₈ H ₃₀ O ₄ S	Triphenyl- methyl sulfate	Triphenyl- methanol-O ¹⁸ and Na ₂ SO ₄ [*]	Alkyl-oxygen bond fission, 93%.	6

References to Table XVIII, 2, HYDROLYSIS OF ESTERS WITH WATER-O¹⁸¹R. Bentley, J. Am. Chem. Soc., 71, 2765 (1949).²E. Blumenthal and J. M. Herbert, Trans. Faraday Soc., 41, 611 (1945).³S. C. Datta, J. N. E. Day and C. K. Ingold, J. Chem. Soc., 1939, 838.⁴M. Polanyi and A. L. Szabo, Trans. Faraday Soc., 30, 508 (1934).⁵I. G. Khaskin, Doklady Akad. Nauk S.S.S.R., 85, 129 (1952); Chem. Abstracts, 46, 10999 (1952).⁶M. Anbar, I. Dostrovsky, D. Samuel and A. D. Yoffe, J. Chem. Soc., 1954, 3603.⁷I. Dostrovsky and F. S. Klein, *ibid.*, 1955, 4401.⁸C. A. Bunton and A. Konasiewicz, *ibid.*, 1955, 1354.

TABLE XVIII, 3

Hydrolysis of Glucosides with Water-O¹⁸¹

Formula	Glucoside	Catalyst	pH	Temp., °C.	Time	Isotopic product
C ₇ H ₁₄ O ₆	α -D-Methyl-glucoside	1.10 N HCl	80	65 hrs.	D-Glucose-1-O ¹⁸
C ₇ H ₁₄ O ₆	α -D-Methyl-glucoside	α -methylglucosidase	6.5	35.7	6 hrs.	D-Glucose-1-O ¹⁸
C ₇ H ₁₄ O ₆	β -D-Methyl-glucoside	1.10 N HCl	80	65 hrs.	D-Glucose-1-O ¹⁸
C ₇ H ₁₄ O ₆	β -D-Methyl-glucoside	almond emulsin	4.6	35.7	24 hrs.	D-Glucose-1-O ¹⁸

¹C. A. Bunton, T. A. Lewis, D. R. Llewellyn, H. Tristram and C. A. Vernon, Nature, 174, 560 (1954).

TABLE XVIII, 4
Exchange of Oxygen-O¹⁸ from Water-O¹⁸ with Organic Compounds

Formula	Compound	Catalyst	Time	Temp., °C.	Oxygen atoms ex.	Notes	Ref.
CO ₂	Carbon dioxide	3 hrs.	room	2	Exchange complete.	2
CH ₄ O	Methanol	0.1 N HCl	24 hrs.	25	0	1
CH ₄ O	Methanol	0.1 N NaOH	48 hrs.	25	0	1
CH ₄ O	Methanol	24 hrs.	25	0	2
CH ₄ N ₂ O	Urea	54 hrs.	25	0	2
C ₂ HCl ₃ O ₂	Trichloroacetic acid	42 hrs.	25	2	Complete exchange.	2
C ₂ H ₃ ClO ₂	Chloroacetic acid	24 hrs.	25	<2	2
C ₂ H ₃ O ₂ K	Potassium acetate	48 hrs.	25	0	1
C ₂ H ₄ O	Acetaldehyde	24 hrs.	25	1	Complete exchange.	2
C ₂ H ₄ O	Acetaldehyde	100 hrs.	20	1	Complete exchange.	3
C ₂ H ₄ O ₂	Acetic acid	0.1 N HCl	40 days	25	2	1
C ₂ H ₄ O ₂	Acetic acid	3 hrs.	100	<2	87.4% complete.	4
C ₂ H ₅ NO ₂	Glycine	72 hrs.	100	8.2% ^a exchange.	5
C ₂ H ₅ NO ₂	Glycine	pH 2	24 hrs.	100	2	Complete exchange.	5
C ₂ H ₅ NO ₂	Glycine	pH 2	70 hrs.	100	2	Complete exchange.	5
C ₃ H ₃ N ₃ O ₃	Cyanuric acid	40 hrs.	100	1.6%.	5
C ₃ H ₆ O	Acetone	24 hrs.	25	0	2
C ₃ H ₆ O	Acetone	24 hrs.	100	Partial exchange.	2
C ₃ H ₉ O ₃	Glycerol	14 days	25	0	2
C ₃ H ₉ O ₃	Glycerol	6 hrs.	100	0	2
C ₄ H ₄ O ₄	Fumaric acid	44 hrs.	25	0	2
C ₄ H ₄ O ₄	Fumaric acid	3-45 hrs.	100	2	6
C ₄ H ₄ O ₄	Maleic acid	20-45 hrs.	100	4	6
C ₄ H ₆ N ₂ O ₂	2,5-Piperazinedione	42 hrs.	100	6.8% ^a exchange.	5
C ₄ H ₆ N ₂ O ₂	2,5-Piperazinedione	48 hrs.	100	0	5

$C_4H_6O_4$	Succinic acid	44 hrs.	25	0	2
$C_4H_6O_4$	Succinic acid	1 hr.	130	2	6
$C_4H_6O_4$	Succinic acid	2-5 hrs.	130	4	6
$C_4H_8N_2O_3$	N-Glycylglycine	24 hrs.	100	0	5
C_4H_8O	2-Butanone	H_2SO_4	b.p.	13	About 94% of equilibrium.
$C_4H_8O_2$	Butyric acid	14 days	25	0	2
$C_4H_{10}O$	Butyl alcohol	0.917 N H_2SO_4	125	12	k (exchange) = 0.56×10^{-6} sec. ⁻¹ .
$C_4H_{10}O$	sec.-Butyl alcohol	0.286 M $HClO_4$	41.2 hrs.	100.8	13	29.3% of equilibrium.
$C_4H_{10}O$	t-Butyl alcohol	0.0913 N H_2SO_4	75	14	k (exchange) = 19.7×10^{-5} sec. ⁻¹ .
$C_6H_{10}O_5$	Xylose	10-100 hrs.	55-100	1	7
$C_5H_{10}O_5$	Arabinose	10-100 hrs.	55-100	1	7
$C_6H_5NO_2$	Nitrobenzene	0.03 N HCl	24 hrs.	25	0	1
$C_6H_5NO_2$	Nitrobenzene	0.03 N NaOH	24 hrs.	25	0	1
C_6H_6O	Phenol	44 hrs.	25	0	2
C_6H_6O	Phenol	24-48 hrs.	100	<1	6
$C_6H_{12}O_6$	Glucose	0-3 hrs.	100	1	6
$C_6H_{12}O_6$	Glucose	390 min.	30	0.3	8	Mutarotation complete.
$C_6H_{12}O_6$	Glucose	120 min.	55	0.8	8	Mutarotation complete.
$C_6H_{12}O_6$	Glucose	45 min.	100	0.9	8	Mutarotation complete.
$C_6H_{12}O_6$	Glucose	$Ca(OH)_2$	18 days	25	2	9	85% fructose.
$C_6H_{12}O_6$	Glucose	$Ca(OH)_2$	10 days	40-55	2	9	87% fructose.
$C_6H_{12}O_6$	Glucose	$Ca(OH)_2$	10 days	40	2	10
$C_6H_{12}O_6$	Glucose	24 hrs.	25	0.5-0.6	10
$C_6H_{12}O_6$	Glucose	$Ca(OH)_2$	24 hrs.	25	0.9-1.0	10
$C_6H_{12}O_6$	Fructose	24 hrs.	25	0.4-0.5	10
$C_6H_{12}O_6$	Fructose	$Ca(OH)_2$	24 hrs.	25	0.6-0.7	10
$C_6H_{12}O_6$	Glucose	10-100 hrs.	55-100	1	7

(Continued)

TABLE XVIII, 4 (Continued)

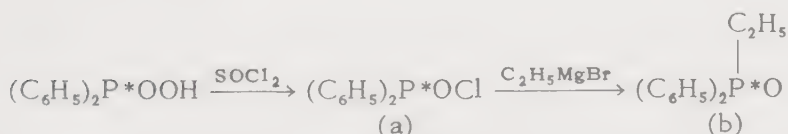
Formula	Compound	Catalyst	Time	Temp., °C.	Oxygen atoms ex.	Notes	Ref.
$C_6H_{12}O_6$	Galactose	...	10-100 hrs.	55-100	1	...	7
$C_6H_{12}O_6$	Fructose	...	10-100 hrs.	55-100	1	...	7
$C_6H_{13}NO_2$	Leucine	...	41 hrs.	100	...	21% ^a exchange.	5
C_7H_6O	Benzaldehyde	...	1-2 hrs.	110	1	...	6
$C_7H_6O_2$	Benzoic acid	...	1-5 hrs.	130	1	...	6
$C_7H_6O_2$	Benzoic acid	...	4 hrs.	100	2	...	1
$C_7H_6O_2$	Benzoic acid	0.1 N HCl	4 hrs.	100	2	...	1
C_7H_7NO	Benzamide	...	23 hrs.	100	0	...	5
$C_7H_{14}O_2$	Amyl acetate	...	44 hrs.	25	0	...	2
$C_8H_6O_4$	Phthalic acid	...	20 hrs.	100	<1	...	6
$C_8H_6O_4$	Terephthalic acid	...	20 hrs.	100	<1	...	6
$C_9H_{11}NO_3$	Tyrosine	...	46 hrs.	100	...	11% ^a exchange.	5
$C_9H_{11}NO_3$	Tyrosine	...	40 hrs.	100	0	...	5
$C_{11}H_{12}Br_2NO_4$	N-Acetyl-3,5-dibromo-L-tyrosine	chymotrypsin	16 hrs.	20	...	67% of equilibrium.	11

^aSamples insufficiently dried.

References to Table XVIII, 4, EXCHANGE OF OXYGEN-O¹⁸ FROM WATER-O¹⁸
WITH ORGANIC COMPOUNDS

- ¹I. Roberts, J. Chem. Phys., 6, 294 (1938).
²M. Cohn and H. C. Urey, J. Am. Chem. Soc., 60, 679 (1938).
³J. M. B. Herbert and I. Lauder, Trans. Faraday Soc., 34, 432 (1938).
⁴R. Bentley, J. Am. Chem. Soc., 71, 2765 (1949).
⁵W. H. Mears and H. Sobotka, *ibid.*, 61, 880 (1939).
⁶M. Koizumi and T. Titani, Bull. Chem. Soc. Japan, 13, 463 (1938); *idem*, 13, 607 (1938).
⁷K. Goto, J. Chem. Soc. Japan, 61, 1283 (1940); Chem. Abstracts, 37, 4055 (1943).
⁸K. Goto and T. Titani, Bull. Chem. Soc. Japan, 16, 403 (1941); Chem. Abstracts, 41, 4435 (1947).
⁹K. Goto, J. Chem. Soc. Japan, 63, 217 (1942); *idem*, 63, 1299 (1942); Chem. Abstracts, 41, 3062 (1947).
¹⁰K. Goto, Bull. Chem. Soc. Japan, 18, 174 (1943).
¹¹D. G. Doherty and F. Vaslow, J. Am. Chem. Soc., 74, 931 (1952).
¹²I. Dostrovsky and F. S. Klein, J. Chem. Soc., 1955, 4401.
¹³C. A. Bunton, A. Konasiewicz and D. R. Llewellyn, *ibid.*, 1955, 604.
¹⁴I. Dostrovsky and F. S. Klein, *ibid.*, 1955, 791.

PHOSPHORUS-32 COMPOUNDS

ETHYLDIPHENYLPHOSPHINE-P³² OXIDE

D. C. Morrison, J. Am. Chem. Soc., 72, 4820 (1950).

A. Procedure (Note 1)

(a) *Diphenylphosphinic-P³² Chloride*. A solution of 0.22 g. (0.001 mole) of diphenylphosphinic-P³² acid in toluene is treated with 0.5 ml. (0.007 mole) of thionyl chloride and refluxed for 1-2 hours. The excess thionyl chloride is removed by distilling about half the toluene, and the remaining solution is cooled and diluted with benzene.

(b) *Ethyldiphenylphosphine-P³² Oxide*. The above solution is then added dropwise to an excess of ethylmagnesium bromide in ether (Note 2), at room temperature. After addition of the phosphinic chloride solution during 1/2 hour, the resulting mixture is refluxed for 1-2 hours, cooled and treated with ice and hydrochloric acid. The organic layer is separated, and the aqueous phase is extracted with ether. The extract is combined with the organic layer, and ether is removed on a water-bath. The residue is then steam-distilled for several hours, cooled, and extracted several times with ether. From the combined extracts, after drying and evaporation of the ether, is obtained a residue which is either crystalline or becomes so on standing. This crude product is decolorized in acetone solution with carbon, concentrated, and recrystallized from ether-petroleum ether or acetone and water.

Several alkyl diarylphosphine oxides prepared by this method from diphenylphosphinic acid and di-*p*-tolylphosphinic acid (Note 3) are presented in Table XIX, 1.

TABLE XIX, 1
 Alkyldiarylphosphine-P³² Oxides

Compound	M.p., °C. (uncor.)	Yield, %
C ₂ H ₅ (C ₆ H ₅) ₂ PO ^a	123-124	67.2
<i>i</i> -C ₃ H ₇ (C ₆ H ₅) ₂ PO ^b	144-146	44.6
<i>i</i> -C ₄ H ₉ (C ₆ H ₅) ₂ PO ^c	132.5-134	75.2
C ₆ H ₅ CH ₂ (C ₆ H ₅) ₂ PO ^d	189-190	69.0
CH ₃ (<i>p</i> -CH ₃ C ₆ H ₄) ₂ PO ^e	145.5-146.5	63.8
C ₄ H ₉ (C ₆ H ₅) ₂ PO	89.5	68.0
C ₄ H ₉ (<i>m</i> -NO ₂ C ₆ H ₄) ₂ PO	124-125.5	68.4

Melting points given in the literature: a, 123 °;¹ b, 145-146 °;² c, 137.5-138 °;² d, 192-193 °;² e, 143 °.³

(c) *Butylbis(-3-nitrophenyl)phosphine-P³² Oxide* (Note 4). The nitrating acid is a mixture of 2-2.5 volumes of sulfuric and 1 volume of fuming nitric acid. Butyldiphenylphosphine oxide, 0.065 g., is added to 10 ml. of this mixed acid at a temperature of 0 °, and the solution is left in an ice-bath for 2 hours. The solution is then poured into water, and the product is extracted with ether. The ether solution is washed repeatedly with water to remove acids and then evaporated. The residue is crystallized from ether-petroleum ether.

B. Notes

1. Apparently the alkyldiarylphosphine oxides described were not prepared with isotopic phosphorus. However, the procedure was developed for that purpose, on a 1 to 10-mmole scale.

2. The Grignard reagent was prepared from 0.25 g. (0.011 mole) of magnesium and 2.5 ml. (0.032 mole) of ethyl bromide.

3. The phosphinic acids used as starting materials were obtained by either of the methods of Kosolapoff.^{4,5} They were recrystallized from water and air-dried. The diphenyl acid melted at 192-193 ° and the di-*p*-tolyl acid at 135.5-136.5 °.

4. By analogy with the case of the nitration of triphenylphosphine oxide, which was shown to yield a *meta*-nitro derivative,⁶ it was assumed that the orientation in this compound was also *meta*.

¹A. Michaelis and H. v. Soden, *Ann.*, 229, 295 (1885).

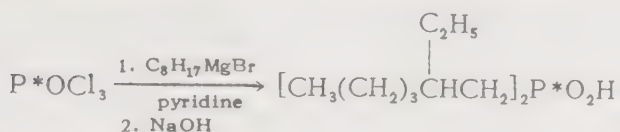
²A. Arbusov, *J. Russ. Phys.-Chem. Soc.*, 42, 395 (1910); *Chem. Abstracts*, 5, 1397 (1911).

³A. Michaelis, *Ann.*, 315, 84 (1901).

⁴G. M. Kosolapoff, *J. Am. Chem. Soc.*, 64, 2982 (1942).

⁵*Idem.*, 71, 369 (1949).

⁶F. Challenger and J. F. Wilkinson, *J. Chem. Soc.*, 125, 2675 (1924).

BIS(2-ETHYLHEXYL)PHOSPHINIC-P³² ACID

W. H. Baldwin and C. E. Higgins, Oak Ridge National Laboratories, unpublished work.

A. Procedure (Note 1)

The apparatus consists of a 300-ml. flask fitted with a reflux condenser, stirrer, dropping funnel and Drierite tubes to protect against moisture. In the flask is placed 4.65 g. (39.4 mmoles) of phosphoryl-P³² chloride, 2.4 g. (30.4 mmoles) of dry pyridine and 50 ml. of dry ether. With stirring, a solution of 2-ethylhexylmagnesium bromide is added over a period of 1 hour. The mixture is then refluxed on a steam-bath, with stirring, for 1 hour. After remaining at room temperature for 16 hours, the reaction mixture is treated with 25 ml. of ice-cold 4 *N* sulfuric acid and stirred until both layers are clear. The aqueous phase is separated, and the ether solution is washed twice with an equal volume of water. The product is extracted from the ether solution into an equal volume of water containing 60 mmoles of sodium hydroxide. The ether phase is then washed with water until the washes no longer become cloudy upon acidification. The combined alkaline solution and wash waters are acidified with 1 *N* sulfuric acid, and the liberated crude product is extracted into carbon tetrachloride. The latter solution is washed free of sulfuric acid with water and dried by azeotropic distillation, and the solvent is removed under reduced pressure. The crude product weighs 3.0 to 5.6 g. (34–63.6%).

The crude product, 2.8 g., is refluxed in a dry atmosphere with an equal weight of phosphorus pentachloride (Note 2) and then fractionated in a micro distilling apparatus¹ at 4 mm. The fraction distilling below 165° is discarded, and the product, 1.53 g., is collected between 165–175° with the heating-bath temperature at 175–185° (Note 3). The bis(2-ethylhexyl)phosphinic-P³² chloride is hydrolyzed with excess alkali, the solution is acidified with mineral acid, and the bis(2-ethylhexyl)phosphinic-P³² acid is extracted into carbon tetrachloride. The extract is washed with water, dried by azeotropic distillation and concentrated to obtain 1.2 g. (10%) of product.

B. Notes

1. An adaptation of the method of Kosolapoff,² as developed by W. M. Whaley, was used in the preparation of this compound. Michaelis and

Wegner³ were the first to employ the Grignard reaction in the synthesis of aromatic phosphinic acids, under conditions which precluded the formation of by-products, by the reaction of arylmagnesium halides with 1-piperidylphosphonic dichloride.

2. According to Kosolapoff,² the product is converted to the corresponding chloride and distilled to eliminate traces of alkylphosphonic acid.

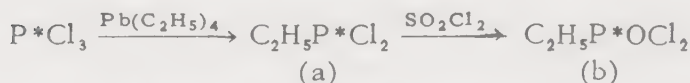
3. The fractions were analyzed by hydrolysis with excess alkali and back titration with standard acid. The first fraction contained 2-ethylhexylphosphonic-P³² dichloride.

¹B. Witten and J. I. Miller, J. Am. Chem. Soc., 70, 3886 (1948).

²G. M. Kosolapoff, J. Am. Chem. Soc., 71, 369 (1949).

³A. Michaelis and F. Wegner, Ber., 48, 316 (1915).

ETHYLPHOSPHONIC-P³² DICHLORIDE



B. C. Saunders and T. S. Worthy, J. Chem. Soc., 1953, 2115.

A. Procedure (Note 1)

(a) *Ethylphosphonous-P³² Dichloride* (Note 2). In a small reaction flask with sealed-on water condenser, stopcock and standard-taper joint (Note 3), is placed 1.25 g. (3.9 mmoles) of tetraethyllead (Note 4). With the apparatus attached to a vacuum manifold, the tetraethyllead is cooled in liquid air, the system is evacuated, and 1.0 ml. (11.4 mmoles) of pure phosphorus-P³² trichloride is distilled into the reaction flask. Dry nitrogen is admitted to the system and, with water in the condenser, the reaction mixture is heated to 110° and stirred magnetically for 72 hours. After cooling, the ethylphosphonous-P³² dichloride is transferred, under vacuum, into a graduated trap. The yield of product, n_D^{25} 1.4900, is 1.15 ml., 1.5 g. (99%).

(b) *Ethylphosphonic-P³² Dichloride*. In an all-glass distillation apparatus (Note 1), with sealed-on vertical condenser, standard-taper joint for connecting to a vacuum manifold, and a side-arm delivery tube below the condenser, is placed 5 ml. of dry benzene. With the apparatus attached to the manifold, the benzene is frozen and the system is evacuated. A tube containing 1.5 ml. of redistilled sulfuryl chloride is then attached to the manifold, and about one-third is evaporated and collected in a trap (Note 5). The remaining sulfuryl chloride, 1.0 ml. (1.67 g., 12 mmoles), is transferred into the distillation apparatus. Then, 1.15 ml. (1.48 g., 11.0 mmoles) of ethylphosphonous-P³² dichloride is transferred, in three portions, into the distillation apparatus, the contents of which

are allowed to melt slowly after each addition. Finally benzene and other volatile materials are removed by continuous evacuation of the system with a water aspirator, *via* two cold traps in series. With the pressure in the system maintained at 20 mm., 1.35 g. (80%) of pure ethylphosphonic- P^{32} dichloride, n_D^{20} 1.4670, is distilled into a small receiver attached to the side-arm.

B. Notes

1. High-vacuum techniques were used throughout this synthesis. Diagrams of the apparatus are shown in Figure XIX, 1.

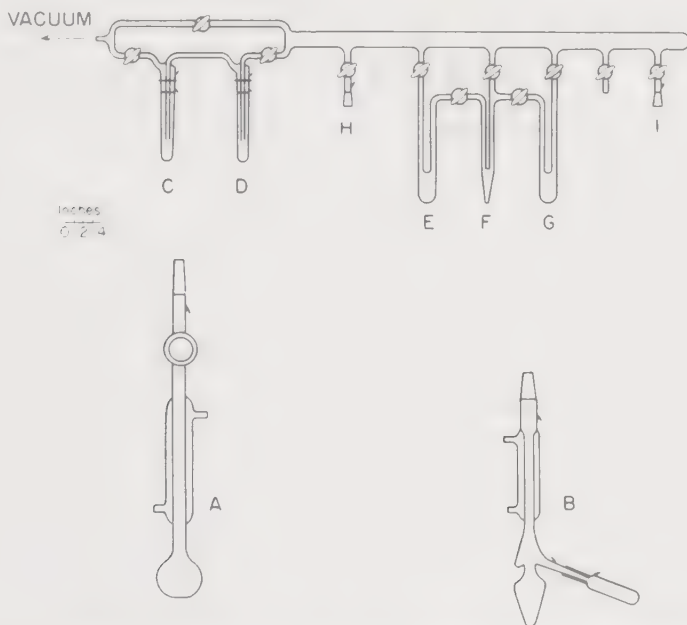


Fig. XIX, 1 Vacuum apparatus for the preparation of ethylphosphonic- P^{32} dichloride (B. C. Saunders and T. S. Worthy). A, reaction flask with sealed-on condenser and standard-taper joint; B, distillation apparatus with sealed-on condenser and standard-taper joint; C and D, traps for collecting volatile materials during evacuation of the system; E, F, and G, traps for fractionation of liquids; H and I, standard-taper joints.

2. The preparation of ethylphosphonous dichloride from tetraethyllead and phosphorus trichloride was an adaptation of the procedure described by Kharasch.¹ The following oxidation with sulfuryl chloride was according to a private communication from the same group.

3. The stopcock and standard-taper joint were sealed to the top of the condenser.

4. The tetraethyllead, b.p. 80° (12 mm.), was twice distilled under nitrogen.

5. This serves to remove volatile impurities.

C. Other Preparations

Ethyl phosphite- P^{32} has been prepared² from phosphorus- P^{32} trichloride and isomerized by treatment with ethyl iodide to obtain diethyl ethylphosphonate- P^{32} .

¹M. S. Kharasch, E. V. Jensen and S. Weinhouse, *J. Org. Chem.*, **14**, 429 (1949).

²A. I. Brodskii and L. L. Chervyatsova, *Doklady Akad. Nauk S.S.S.R.*, **90**, 545 (1953).

PHOSPHORYL- P^{32} CHLORIDE (Phosphorus- P^{32} Oxychloride)

METHOD I



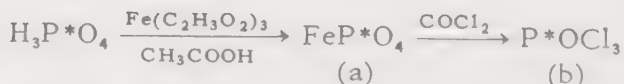
J. L. Kalinsky and A. Weinstein, *J. Am. Chem. Soc.*, **76**, 5882 (1954).

A. Procedure

An aqueous solution of phosphoric- P^{32} acid, 0.08 mmole (10 mc.), in a 25-ml., two-necked flask, is evaporated under vacuum until the residue weighs 0.224 g. (Note 1). The flask is then equipped with a double surface condenser, protected by a drying tube, and a micro dropping funnel and is cooled in a Dry Ice-acetone bath. In the funnel is placed 2.70 g. (12.9 mmoles) of phosphorus pentachloride. The reaction is initiated by adding a small amount, about 0.1 g., of phosphorus pentachloride, removing the freezing bath and allowing the mass to thaw slightly. This process is repeated until the reaction is controllable without freezing; the remainder of the phosphorus pentachloride is then added rapidly.

After the reaction mixture warms to room temperature, it is cautiously refluxed for 15 minutes. After the flask cools, it is immersed in a Dry Ice-acetone bath, and warm water is circulated through the condenser to aid in distilling product from the condenser into the flask. With the reaction flask in a bath at -5° , the phosphoryl- P^{32} chloride is distilled under vacuum; yield, 1.89 g. (96.7%) (Note 2).

METHOD II



J. E. Gardiner and B. A. Kilby, *J. Chem. Soc.*, 1950, 1769.

A. Procedure

(a) *Ferric Phosphate- P^{32}* . Phosphoric- P^{32} acid solution (1.14 mc. in 0.54 ml.) is washed with 20 ml. of phosphoric acid solution (31.92 g./l.)

and water into a 100-ml. graduate (Note 3). The remainder of the solution, 99 ml., is added slowly with vigorous stirring to 50 ml. of ferric acetate solution in acetic acid (Note 4). Solid ammonium acetate, 5 g., is then added, and the mixture is boiled for 5 minutes. The precipitate of ferric phosphate- P^{32} is collected by centrifugation, washed once with ethanol and twice with ether, dried at 120° , powdered with a glass rod and transferred into a quartz tube. After 90 minutes at red heat, the voluminous precipitate shrinks to one-third of its original volume. When cool, the granular powder is packed into a silica tube (10 cm. \times 1 cm. i.d.), with a glass wool plug at each end.

(b) *Phosphoryl- P^{32} Chloride*, (*Phosphorus- P^{32} Oxychloride*). The quartz tube containing the ferric phosphate- P^{32} is connected to the apparatus (Figure XIX, 2) which is comprised of a metering device for supplying a controlled flow of either nitrogen or carbonyl chloride and a series of traps for removing ferric chloride, product, and excess carbonyl chloride from the gas stream. The last two traps contain soda lime and water, respectively. The quartz tube is heated in a furnace at $400\text{--}450^{\circ}$, and the adjoining U-tube trap, packed with glass wool, is maintained at $120\text{--}130^{\circ}$ in a metal-bath (Note 5). After the apparatus is dried with a

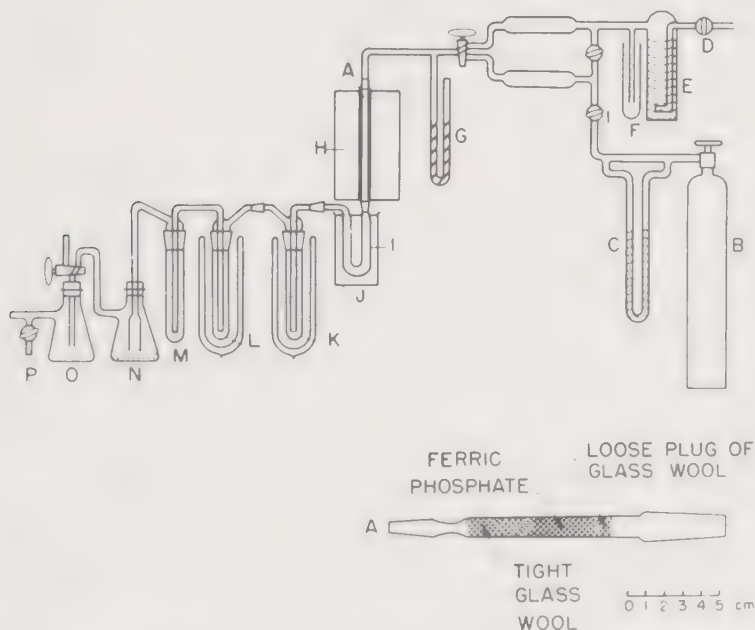


Fig. XIX, 2 Apparatus for the preparation of phosphoryl- P^{32} chloride (J. E. Gardiner and B. A. Kilby). A, quartz tube containing ferric phosphate- P^{32} ; B, carbonyl chloride tank; C, flow meter; D, stopcock for admitting dry air or nitrogen; E, wash bottle containing concentrated sulfuric acid; F, safety valve; G, manometer; H, electric tube furnace; I, U-tube packed with glass wool; J, metal bath; K and L, cold traps equipped with close-fitting brass shields; M, trap containing water; N, trap containing soda lime; O, water bubbler; P, controlled air leak; Q, connection to water aspirator.

flow of dry nitrogen, the two product traps are cooled in Dry Ice-alcohol mixture. With a slight vacuum on the system, a slow stream of carbonyl chloride mixed with air is admitted. As reaction proceeds, the air supply is stopped and the carbonyl chloride stream is increased. About 50 ml. of liquid carbonyl chloride is collected during 6 hours (Note 6). The apparatus is flushed with air, and the tube from the first trap is connected to a simple distilling head. Most of the carbonyl chloride distills off at room temperature, and the final traces are removed with a water-bath at 75°. The phosphoryl-P³² chloride is then vacuum-distilled with liquid air-cooling of the receiver. The yield is 0.93 g. (93%).

B. Notes

1. The residue is largely water, 0.22375 g. (12.4 mmoles). It is possible to reduce the amount of water still further but impractical and unnecessary in most instances.
2. The radiochemical yield was 96%.
3. A 1-ml. aliquot was withdrawn for assay.
4. The solution contained 8.25 g. of iron and 120 ml. of acetic acid per liter.
5. This trap condenses and collects ferric chloride.
6. The flow of carbonyl chloride need not be this fast, according to the authors.

C. Other Preparations

Phosphoryl-P³² chloride has been prepared from disodium phosphate-P³², in 80% yield,¹ using a procedure similar to that reported by Lindberg;² by the oxidation of phosphorus-P³² trichloride with potassium chlorate;³ by the passage of carbonyl chloride over heated calcium phosphate-P³²-charcoal;⁴ by the action of phosphorus pentachloride on anhydrous phosphoric-P³² acid^{5,6} and by the reaction of silver phosphate-P³² and phosphorus pentachloride in a sealed tube at 130°.⁶

¹B. Axelrod, *J. Biol. Chem.*, 176, 295 (1948).

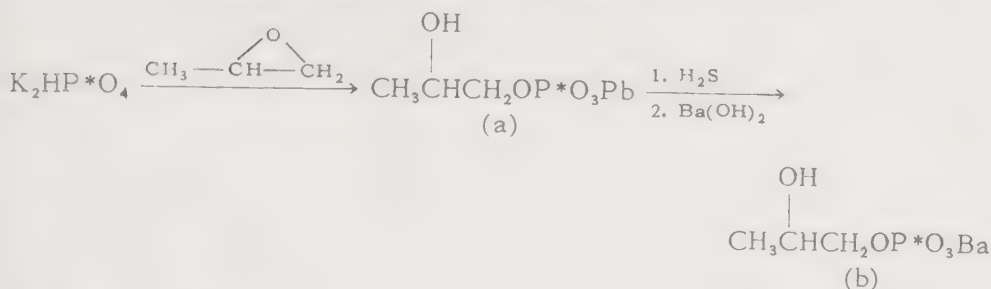
²O. Lindberg, *Arkiv. Kemi, Mineral. Geol.*, 23A, No. 2, (1946).

³E. Chargoff, *J. Am. Chem. Soc.*, 60, 1700 (1938).

⁴T. E. Banks, J. C. Bournsnel, H. M. Dewey, G. E. Francis, R. Tupper and A. Wormald, *Biochem. J.*, 43, 518 (1948).

⁵W. H. Baldwin and C. E. Higgins, Oak Ridge National Laboratory, unpublished work.

⁶D. H. Murray and J. W. T. Spinks, *Can. J. Chem.*, 30, 497 (1952).

BARIUM 2-HYDROXYPROPYL PHOSPHATE- P^{32} 

H. Rudney, J. Biol. Chem., 210, 353 (1954).

A. Procedure (Note 1)

(a) *Lead 2-Hydroxypropyl Phosphate- P^{32}* . Phosphoric- P^{32} acid is converted to the dipotassium salt by titration with potassium hydroxide to the phenolphthalein end-point. The solution is evaporated to dryness, and to 0.3484 g. (2 mmoles) of dipotassium phosphate- P^{32} is added 1.7424 g. (30 mmoles) of propylene oxide and sufficient water to make the final volume of solution 5 ml. In a tightly stoppered flask, the mixture is kept at room temperature for 3 days with occasional mixing. Then, the solution is adjusted to pH 7.6 with nitric acid, and 2-hydroxypropyl phosphate is precipitated as the lead salt by the addition of a saturated basic lead acetate solution, in slight excess. After several hours, the mixture is centrifuged and the precipitate of lead 2-hydroxypropyl phosphate- P^{32} is washed with water (Note 2).

(b) *Barium 2-Hydroxypropyl Phosphate- P^{32}* . The lead salt, suspended in 5 ml. of water, is treated with hydrogen sulfide; the mixture is centrifuged, and the precipitate of lead sulfide is washed with 2 ml. of water. The combined solution and wash water are freed of hydrogen sulfide by aeration, the pH is adjusted to 10.2 with barium hydroxide solution, and any precipitate is removed by centrifugation. To the supernatant liquid is added 4 volumes of 95% alcohol, and a flocculent white precipitate of barium 2-hydroxypropyl phosphate- P^{32} is formed. After the mixture is cooled for 4 hours, the precipitate is collected and washed successively with the following solutions: 100% alcohol, 75% alcohol-25% ether, 50% alcohol-50% ether, 25% alcohol-75% ether, and 100% ether. After the product is dried *in vacuo* over phosphorus pentoxide, the yield is 50%.

B. Notes

1. Barium 2-hydroxypropyl phosphate- P^{32} was synthesized, using modifications of the procedures of Atherton¹ and Lampson.² In a new method³ for the synthesis of glucose-6-phosphate, 5,6-anhydro-1,2-O-isopropylidene-D-glucofuranose was heated with aqueous dipotassium phosphate. Since

the phosphate ester of the primary alcohol group was formed on cleavage of the anhydro ring structure, it was assumed that 2-hydroxypropyl phosphate was formed from propylene oxide and the same reagent.

2. The lead salt had a purity of 70-80% at this point.

C. Other Preparations

Sodium 2-hydroxypropyl phosphate- P^{32} has been prepared⁴ from 1,2-propanediol and phosphoryl- P^{32} chloride. The solution containing 2-hydroxypropyl phosphate- P^{32} was neutralized with sodium hydroxide, and the product was precipitated by addition of alcohol and ether.

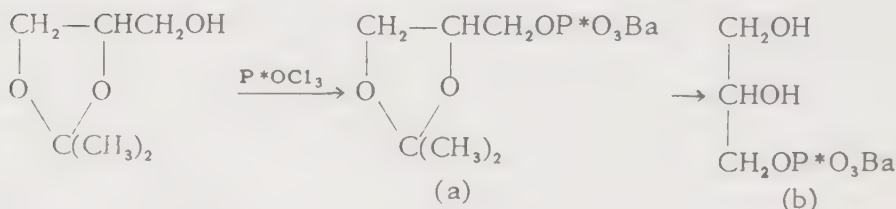
¹F. R. Atherton, H. T. Openshaw and A. R. Todd, J. Chem. Soc., 1945, 382.

²G. P. Lampson and H. A. Lardy, J. Biol. Chem., 181, 697 (1949).

³*Idem*, 181, 693 (1949).

⁴O. Lindberg, Arkiv Kemi, 24, 1 (1946).

BARIUM 2,3-DIHYDROXYPROPYL PHOSPHATE- P^{32}



E. Chargaff, J. Am. Chem. Soc., 60, 1700 (1938).

A. Procedure (Note 1)

(a) *Barium 2,2-Dimethyl-1,3-dioxolane-4-methyl Phosphate- P^{32}* . During 30 minutes, 13.2 g. (0.1 mole) of 1,2-*O*-isopropylideneglycerol is added dropwise to a stirred mixture of 15.3 g. (0.1 mole) of phosphoryl chloride and 50 ml. of dry quinoline, cooled to -20° . After the mixture remains at -20° for 1 hour, it is slowly warmed to room temperature and added, with stirring, to 800 ml. of ice-water which has been acidified with 70 ml. of 25% sulfuric acid. The solution is treated with silver carbonate, and the silver chloride is collected on a matt of activated carbon. The filtrate is treated with hydrogen sulfide to remove excess silver and again filtered. To the clear filtrate is added 100 g. of finely pulverized barium hydroxide, with stirring. To remove excess barium the solution is treated with carbon dioxide and filtered through a bed of carbon. The filtrate is evaporated to dryness with reduced pressure and a water-bath temperature of 40° (Note 2). The colorless residue is shaken with 800 ml. of cold water, filtered (Note 3) and evaporated under reduced pressure, at 40° , to a volume of about 120 ml. Barium 2,3-isopropylidenedioxypropyl phosphate begins to crystallize, and 120 ml. of alcohol is added to the

solution. After cooling the mixture at 0°, the yield of product is 33 g. (82%) (Note 4).

(b) *Barium 2,3-Dihydroxypropyl Phosphate-P³²*. To a solution of 5 g. of barium 2,3-isopropylidenedioxypropyl phosphate in 130 ml. of hot water is added 35 ml. of 1 N sulfuric acid, and the mixture is heated for 15 minutes on a boiling water-bath. Then the mixture is quickly cooled, treated with 7 g. of crystalline barium hydroxide, saturated with carbon dioxide and filtered. The filtrate is evaporated to dryness at 10-15 mm. pressure and a temperature of not more than 40°, and the amorphous residue is dissolved in water. After the solution stands several days the product crystallizes slowly (Note 5). The yield of air-dried product, which does not contain water, is 3.5 g. (90%) (Note 6).

B. Notes

1. The synthesis of barium 2,3-dihydroxypropyl phosphate-P³² was according to the procedure of Fischer and Pfähler.¹ The experimental details given are from their work.

2. The quinoline is all removed with the water.

3. All the residue dissolves except a trace of barium carbonate.

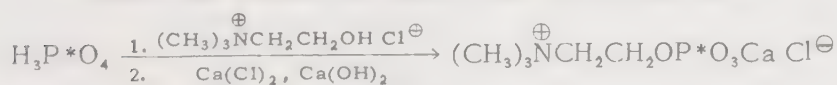
4. The salt crystallizes with 3 moles of water. The solubility of the anhydrous salt in water at 25° is 3.64 g. per 100 ml. It is practically insoluble in most organic solvents.

5. Crystallization may be hastened by heating the solution.

6. A saturated aqueous solution at 22° contains 1.3% of barium 2,3-dihydroxypropyl phosphate. Chargaff obtained the barium salts of both the isotopic compounds in crystalline form.

¹E. Fischer and E. Pfähler, Ber., 53, 1606 (1920).

CALCIUM CHOLINE CHLORIDE PHOSPHATE-P³²



R. F. Riley, J. Am. Chem. Soc., 66, 512 (1944).

A. Procedure (Note 1)

A solution of 150 mg. of phosphoric-P³² acid in 6 ml. of water is placed in a 35-ml. flask equipped with two outlets attached to stopcocks. The dilute acid is concentrated until anhydrous by immersion of the flask in an oil-bath, which is gradually heated to 180°, while a stream of dry air is passed through the flask (Note 2). Then, 214 mg. of dry choline chloride is well mixed with the anhydrous acid. The mixture is then maintained at 165° for 12 hours, under vacuum, in the presence of a mix-

ture of phosphorus pentoxide and asbestos contained in a Soxhlet thimble. After cooling, the mixture is dissolved in a few ml. of water, 100 mg. of calcium chloride is added, and the solution is adjusted to the phenolphthalein end-point with saturated calcium hydroxide solution. The solution is concentrated, filtered to remove calcium carbonate and diluted with an equal volume of absolute alcohol. The crystalline product is collected, washed with 60%, 80% and absolute alcohol, and dried (Note 3). The yield is 306 mg. (63%) (Notes 4 and 5).

B. Notes

1. The published procedures for the synthesis of the monophosphoric acid ester of choline are not well suited to incorporation of phosphorus-P³². They either permit extensive isotopic dilution¹⁻³ or yield a product contaminated by diester and inorganic phosphate.⁴

2. Only 0.005% of the isotope was lost during the evaporation.

3. This isolation procedure is according to Plimmer and Burch.³

4. The unreacted phosphate-P³² was recovered as magnesium ammonium phosphate, making the total recovery of isotope 96%.

5. By the isotopic dilution method of analysis, the product was found to contain 0.02% of contaminating inorganic phosphorus.

¹F. Inukai and W. Nakahara, *Proc. Imp. Acad. (Tokyo)*, **11**, 260 (1935).

²A. B. L. Beznak and E. Chain, *Quart. J. Exp. Physiol.*, **26**, 201 (1936-1937).

³R. H. A. Plimmer and W. J. N. Burch, *Biochem. J.*, **31**, 398 (1937).

⁴E. L. Jackson, *J. Am. Chem. Soc.*, **57**, 1903 (1935).

4-NITROPHENYL DIHYDROGEN PHOSPHATE-P³²



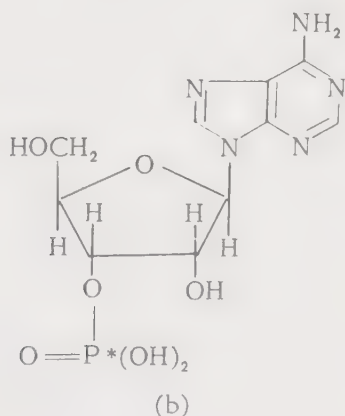
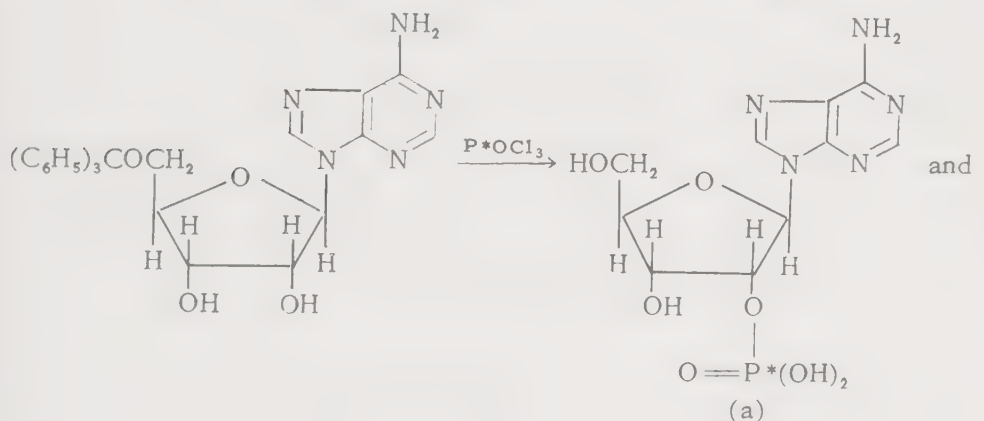
B. Axelrod, *J. Biol. Chem.*, **176**, 295 (1948).

Procedure

With good stirring and cooling, 1.0 ml. of phosphoryl-P³² chloride is added to 310 mg. of 4-nitrophenol, dissolved in 1.4 ml. of dry chloroform, and followed by 0.34 ml. of dry pyridine. After 30 minutes, several pieces of ice are added, and the reaction mixture is set aside for several hours to insure decomposition of the acid chloride. Extraction of the reaction mixture with chloroform and removal of the solvent in a stream of air leave the crude product as a sticky tar. The crystalline salt, disodium 4-nitrophenyl phosphate-P³², is obtained by adding to the residue 2 ml. of water and enough sodium ethylate to make the solution just alkaline to phenolphthalein, followed by a large excess of 1:1 acetone and ethanol.

The crystals, which are collected and washed with ethanol-ether (1:1), weigh 100 mg. For further purification, the product is converted to the barium salt. The sodium salt is dissolved in 3 ml. of water, the solution is made slightly basic to phenolphthalein, and a slight excess of barium acetate is added. A slight precipitate is removed by centrifugation, and the supernatant liquid is diluted with ethanol until concentration of the latter is 78%. The resulting precipitate of barium 4-nitrophenyl phosphate- P^{32} is washed twice with small volumes of 95% ethanol and finally with ether.

ADENOSINE DIHYDROGEN 3'-PHOSPHATE- P^{32}



G. R. Barker, J. Chem. Soc., 1954, 3396.

A. Procedure

(a) *Adenosine dihydrogen 2'-phosphate- P^{32}* . To a hot solution of 1.03 g. of adenosine in 30 ml. of dry pyridine is added 2.14 g. of triphenylchloromethane. The solution is kept for 3 hours at $95-100^\circ$, with

exclusion of moisture, and is then cooled to 0° (Note 1). To this solution is added, with stirring, 0.75 ml. of phosphoryl- P^{32} chloride (Note 2) and 2 ml. of pyridine containing 0.075 ml. of water. The solution is set aside at room temperature overnight and is then cooled to 0° . With stirring, 10 ml. of a 50% aqueous solution of pyridine, cooled to 0° , is added, and the entire solution is poured into 400 ml. of water. Saturated aqueous barium chloride solution is added dropwise to coagulate the precipitate, which is collected by centrifugation and washed twice with water. The precipitate is refluxed with 70 ml. of 80% aqueous acetic acid for one-half hour, and the resulting solution is cooled, filtered and poured into 500 ml. of water with stirring. The precipitated triphenylmethanol is collected on a bed of charcoal, and the pH of the filtrate is adjusted to 7.5 with hot, saturated barium hydroxide solution (Note 3). The solution is diluted to 10 l. and percolated through Dowex-1 ion-exchange resin in the formate form (10×4.5 cm. column; mesh size 200-400). The column is washed free of barium ions with water and is then eluted first with 1 l. of 0.02 *N* formic acid and then with 0.15 *N* formic acid. The optical density at $260\text{ m}\mu$ of 100-ml. fractions, collected every 15 minutes, is determined. Fractions containing adenosine-2' dihydrogen phosphate- P^{32} are combined, concentrated below 40° to 100 ml., and freeze-dried. The yield of product, m.p. 187° (dec.), is 0.203 g. (Note 4).

(b) *Adenosine dihydrogen 3'-phosphate- P^{32}* . Fractions containing adenosine-3' dihydrogen phosphate- P^{32} are combined, and the product is isolated in the manner described above. The yield of material melting at 195° (dec.) is 0.132 g. (Note 5).

B. Notes

1. This solution of $O^{5'}$ -trityladenosine and $N^6, O^{5'}$ -ditrityladenosine was treated with phosphoryl- P^{32} chloride without isolation of the intermediate, but details are given for the isolation of $O^{5'}$ -trityladenosine, m.p. 260° with sintering from 254° , and $N^6, O^{5'}$ -ditrityladenosine, m.p. $200-202^{\circ}$.

2. The phosphoryl- P^{32} chloride was vacuum-distilled before use. The glass system used in introducing the isotope is shown diagrammatically by Barker, and the procedure is given in detail.

3. A sample of this solution was diluted to 250 ml. (optical density 0.6 at $260\text{ m}\mu$) and percolated through Dowex-1 resin (30×1 cm.). The column was eluted in the manner described, and 6-ml. fractions were collected every 45 minutes. The first 64 fractions had zero optical density at $260\text{ m}\mu$; adenosine dihydrogen 2'-phosphate- P^{32} was collected in 16 fractions; a further 47 fractions showed no absorption, and adenosine-3' hydrogen phosphate- P^{32} was then collected in 30 fractions.

4. The product gave one spot (R_f 0.74) on a paper chromatogram developed with a mixture of 5% aqueous disodium phosphate and isoamyl alcohol.¹

5. This material was also chromatographically homogeneous (R_f 0.67) using the same solvent system as above.

C. Other Preparations

Adenosine triphosphate- P^{32} has been prepared^{2,3} by an enzymatically catalyzed exchange between inorganic pyrophosphate- P^{32} and adenosine triphosphate. Adenyl acetate was also converted to adenosine triphosphate- P^{32} in the presence of the enzyme.²

Adenosine dihydrogen 5'-phosphate- P^{32} has been prepared⁴ from adenosine triphosphate and potassium dihydrogen phosphate- P^{32} in a system containing yeast extract (Lebedev juice). Deamination of the adenosine dihydrogen 5'-phosphate- P^{32} gave inosine dihydrogen 5'-phosphate- P^{32} , and hydrolysis with hydrochloric acid solution gave ribose dihydrogen 5'-phosphate- P^{32} .

¹C. E. Carter, J. Am. Chem. Soc., 72, 1466 (1950).

²P. Berg, *ibid.*, 77, 3163 (1955).

³M. E. Jones, F. Lipmann, H. Hilz and F. Lynen, *ibid.*, 75, 3285 (1953).

⁴L. V. Eggleston, Biochem. J., 58, 503 (1954).

DIBUTYL HYDROGEN PHOSPHATE- P^{32}



C. E. Higgins and W. H. Baldwin, Oak Ridge National Laboratories, unpublished work.

A. Procedure

Anhydrous sodium acetate (Note 1), 3.94 g. (48 mmoles), and 15.72 g. (59 mmoles) of butyl phosphate- P^{32} (Note 2) are mixed in a large test tube (2.3×15 cm.). A cold-finger condenser is inserted into the tube, and the mixture is heated in an oil-bath at 200° for 3 hours. After cooling, the reaction mixture is transferred to a separatory funnel with 100 ml. of water. The aqueous solution is adjusted to pH 7-8 with 0.1 N sodium hydroxide (Note 3). Butyl acetate and excess butyl phosphate- P^{32} are removed by extraction of the basic solution with 4 portions of carbon tetrachloride (Note 4). The aqueous solution is acidified with 1 N hydrochloric acid and extracted with 4 portions of carbon tetrachloride which are combined and washed once with water (Note 5). The carbon tetrachloride layer is dried by azeotropic distillation and evaporated un-

der reduced pressure. After 5 hours at 2 mm. pressure, the product weighs 9.0 to 9.5 g. (89-94%).

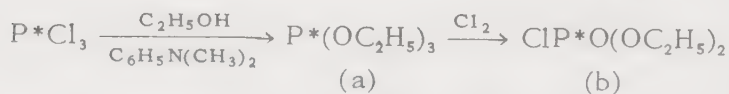
B. Notes

1. The sodium acetate was dehydrated at 300° for at least 2 hours.
2. Excess ester was used as solvent for the reaction. However, when 1 mole of ester was reacted with 2 moles of sodium acetate, under similar conditions, a 90% yield of dibutyl hydrogen phosphate of equal purity was obtained.
3. Wide range pH paper is suitable.
4. The excess phosphate ester recovered from the extract may be recycled, yielding a product that is as pure as the first.
5. The volume of water was equal to one-fourth the volume of carbon tetrachloride.

C. Other Preparations

Higgins and Baldwin have also found that de-esterification of ternary phosphate esters is effected by amines. Heating butyl phosphate with a five-fold excess of ethanolamine produces dibutyl hydrogen phosphate in high yield.

ETHYL PHOSPHOROCHLORIDATE-P³²



K. Gardner and D. F. Heath, *Anal. Chem.*, 25, 1849 (1953).

A. Procedure (Note 1)

(a) *Ethyl Phosphite-P³²*. According to the following example,¹ 151.2 g. of phosphorus trichloride, dissolved in an equal volume of dry ether, is added slowly to a mixture of 151.8 g. of ethyl alcohol and 399.3 g. of dimethylaniline (Note 2) dissolved in 200 ml. of dry ether. The reaction is protected from moisture with a calcium chloride tube. The mixture is well mixed and kept cold by immersion in water. Finally, the mixture sets to a nearly solid mass which is kept at room temperature for 2 hours. The amine hydrochloride is collected, and the filtrate is dried over sodium sulfate. After removal of the ether, distillation of the residue yields 124 g. (73%) of product, b.p. 48° (12.5 mm.).

(b) *Ethyl Phosphorochloridate-P³²*. Dry chlorine is passed through 16.6 g. (0.1 mole) of ice-cold ethyl phosphite until a faint permanent green color is produced (Note 3). Excess chlorine is removed by bubbling

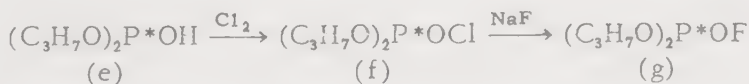
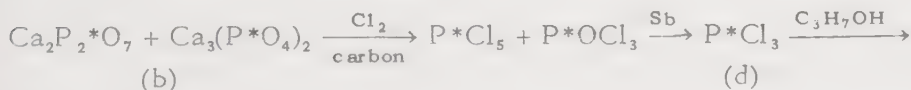
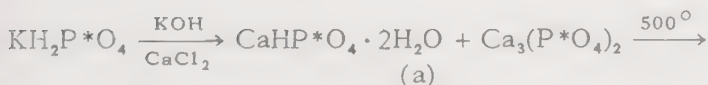
dry air through the reaction mixture under reduced pressure for 3 hours (Note 4). After treatment with lead carbonate and filtration through kieselguhr, the product is distilled. The yield of material, b.p. 93–95° (18 mm.), is 13.7 g. (80%).

B. Notes

1. The procedure used was an adaptation of that of McCombie;¹ no experimental details were given.
2. Pyridine may also be used.
3. This occurs when the gain in weight is equal to that theoretically required.
4. No product was lost in this step.

¹H. McCombie, B. C. Saunders and G. J. Stacey, J. Chem. Soc., 1945, 380.

ISOPROPYL PHOSPHOROFUORIDATE-P³² (Isopropyl Fluorophosphate-P³²)



B. Witten and J. I. Miller, J. Am. Chem. Soc., 70, 3886 (1948).

A. Procedure

(a) *Calcium Hydrogen Phosphate-P³² and Calcium Phosphate-P³²*. A mixture of 1 g. of potassium dihydrogen phosphate-P³² (Note 1) and 0.412 g. of potassium hydroxide is dissolved in 3.5 ml. of water. This solution is added, with stirring, to a 15-ml. centrifuge tube containing 2.2 g. of calcium chloride dissolved in 7 ml. of water. The mixture is centrifuged, and the precipitate of calcium hydrogen phosphate-P³² with some calcium phosphate-P³² is washed three times with 3-ml. portions of water (Note 2).

(b) *Calcium Pyrophosphate-P³² and Calcium Phosphate-P³²*. The tube containing the moist calcium hydrogen and calcium phosphates-P³² is heated at 500° for 3 hours in an electric furnace to convert the calcium hydrogen phosphate-P³² to calcium pyrophosphate-P³².¹

(c) *Phosphorus-P³² Pentachloride and Phosphoryl-P³² Chloride*. A quartz tube, 15 mm. i.d. × 60 cm., is ground to fit a 25-ml. standard-taper flask with a side-tube, to which is attached a drying tube containing Drierite.

The calcium phosphate- P^{32} —pyrophosphate- P^{32} mixture, 0.93 g., is intimately mixed with 2.5 g. of 30-60 mesh charcoal and introduced into the quartz tube. The tube, held in a horizontal position, is heated by an electric furnace at 700° . While the mixture is heated, a stream of chlorine is passed through the tube at a flow rate of 20-25 ml. per minute, and the receiver is cooled in a Dry Ice-bath.¹⁻³ After 6 hours, the exposed part of the tube is heated with a free flame to volatilize any product remaining in the tube. The yield of product, consisting of a mixture of phosphorus- P^{32} pentachloride and phosphoryl- P^{32} chloride, is 1.5 g.

(d) *Phosphorus- P^{32} Trichloride*. Phosphorus pentachloride is reduced to the trichloride with antimony.^{1,4-6} The apparatus consists of a reaction flask connected to a quartz tube (4 mm. i.d. \times 19 cm.), into which is introduced 0.35 g. of 30-60 mesh charcoal. The tube, held in a horizontal position, is also attached to a 5-ml. two-necked receiver, containing a glass-enclosed magnetic stirrer and equipped with a water condenser and Drierite tube.

The reaction flask, containing 1.5 g. of phosphorus- P^{32} pentachloride-phosphoryl- P^{32} chloride mixture, is cooled in a Dry Ice-bath, and 0.9 g. of powdered antimony is sprayed over the mixture (Note 3). With the quartz tube heated in a furnace at 650° , the Dry Ice-bath is removed, and the reaction flask is warmed until the reaction (Note 4) between the pentachloride and the antimony begins. When the vigorous reaction has subsided, the mixture is heated with an oil-bath; the temperature is gradually raised to 150° over a period of 90 minutes. Phosphoryl chloride is reduced to the trichloride as the vapors pass over the hot carbon in the tube^{3,6} (Note 5). The yield of phosphorus- P^{32} trichloride, b.p. $76-78^{\circ}$, is 0.81 g. (80%).

(e) *Diisopropyl Hydrogen Phosphite- P^{32}* (Note 6). Prior to the synthesis of phosphorus- P^{32} trichloride as described above, 1.37 g. of anhydrous isopropyl alcohol is placed in the 5-ml. receiver. The alcohol is stirred vigorously with the magnetic stirrer and cooled in an ice-bath as the trichloride is generated. After all the trichloride is generated, the temperature is maintained at 0 to 10° for 20 minutes. The ice-bath is then removed, and the temperature of the reaction mixture is allowed to rise to 25° .

(f) *Isopropyl Phosphorochloridate- P^{32} , (Isopropyl Chlorophosphate- P^{32})* (Note 6). A gas inlet tube is introduced into the flask containing the diisopropyl hydrogen phosphite- P^{32} , above. The flask is cooled to 0° , the system is evacuated to 450 mm., and a stream of dry chlorine precooled to -25° is introduced into the flask at a flow rate of about 200-400 ml. per minute. Chlorine is bubbled through the reaction mixture until the colorless solution becomes light green and this color persists for at least 5 minutes (Note 7). Then the chlorine is replaced by a slow stream of nitrogen and the pressure is reduced to 50 mm. The

temperature of the reaction mixture is maintained at 0° for 30 minutes and at 5° for 30 minutes. The pressure is then reduced to 35 mm., and the temperature is kept at 60° for 30 minutes (Note 8). The flask is connected to a micro still (Note 9), and the fraction distilling at 6 mm. with a bath temperature of 85° and a column temperature of 90° is collected in a 5-ml. two-necked flask. The yield of product is 0.6 g.

(g) *Isopropyl Phosphorofluoridate- P^{32} , Isopropyl Fluorophosphate- P^{32}* (Note 6). To the two-necked flask, containing 0.6 g. of isopropyl phosphorochloridate- P^{32} are added 1.0 g. of anhydrous sodium fluoride, 1 ml. of dry carbon tetrachloride and a glass-enclosed magnetic stirring bar. A condenser and drying tube are attached to the flask, and the mixture is refluxed for 5 hours with vigorous stirring. The reaction flask is connected to the micro still (Note 8), and carbon tetrachloride is removed at 200 mm. pressure. Isopropyl phosphorofluoridate- P^{32} is distilled at 8 mm. with a bath temperature of 80° and a column temperature of 85° . The yield of product is 0.45 g. (33% based on potassium dihydrogen phosphate- P^{32}).

B. Notes

1. If phosphoric- P^{32} acid was used, potassium monohydrogen phosphate was added as carrier.

2. The excess calcium chloride is removed.

3. The antimony, 20-mesh and finer, is rapidly sprayed from an eye dropper in such a manner as to completely cover the surface of the chloride mixture.

4. It may become necessary to moderate the exothermic reaction with an ice-bath.

5. When the hot charcoal treatment was omitted, the product boiled over a range of 90 – 100° , indicating the presence of phosphoryl chloride.

6. The procedures used in the preparation of phosphorus labeled diisopropyl hydrogen phosphite, isopropyl phosphorochloridate and isopropyl phosphorofluoridate are adaptations of methods described earlier.⁷⁻⁹

7. Further chlorination results in a decrease in yield. The chlorination was carried out in a slight vacuum in order to remove hydrogen chloride as rapidly as possible, since its presence also resulted in a lower yield.

8. This procedure effects removal of last traces of hydrogen chloride.

9. The micro column, which is a modification of that described by Craig,¹⁰ is shown in Figure XIX, 3.

C. Other Preparations

Phosphorus- P^{32} trichloride has been prepared directly from phosphorus- P^{32} and chlorine gas, by Saunders and Worthy,¹¹ in about 90% yields. The

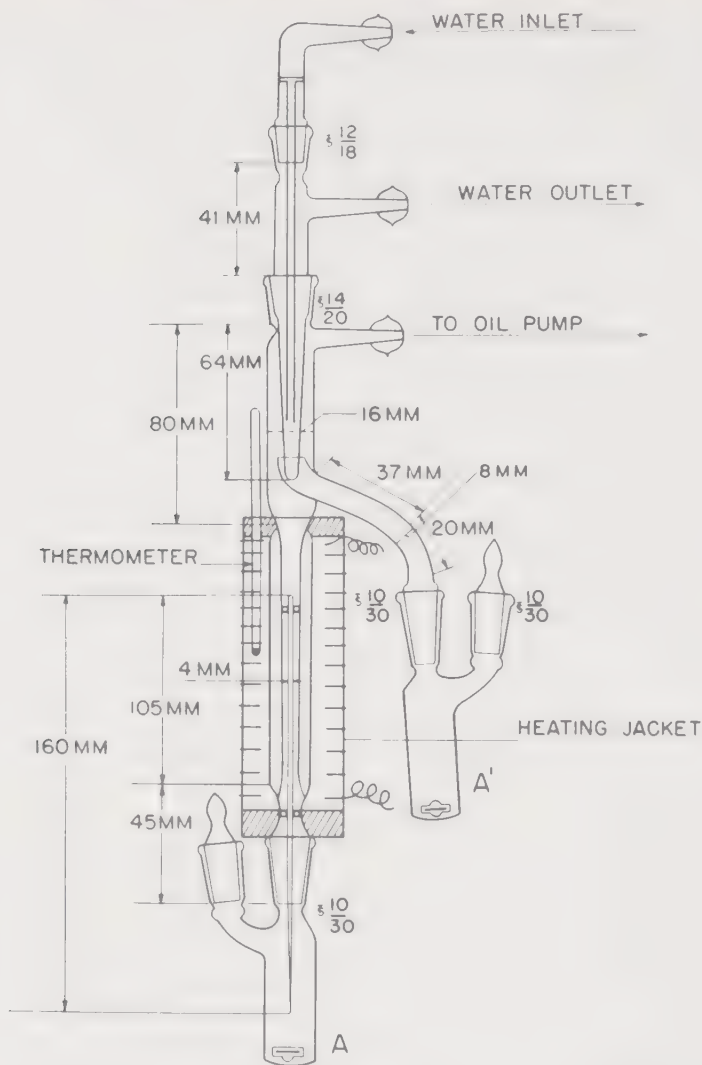


Fig. XIX, 3 Micro still used in the preparation of isopropyl phosphorofluoridate- P^{32} (B. Witten and J. I. Miller). A and A', 5-ml. flasks used as reaction vessels, distillation flasks and receivers:

product contained some free phosphorus- P^{32} and phosphorus- P^{32} pentachloride. Formation of the latter compound in greater amount could only be prevented by introduction of chlorine at a very low rate.

Diisopropyl hydrogen phosphite- P^{32} , isopropyl phosphorochloridate- P^{32} and isopropyl phosphorofluoridate- P^{32} have been prepared¹¹ by procedures similar to those described.

¹J. W. Mellor, *A Comprehensive Treatise on Inorganic and Theoretical Chemistry*, Vol. III, Longmans, Green and Co., New York.

²Gay-Lussac and Thenard, *Recherches Physico-Chimiques*, Paris, 2, 176 (1811).

³B. A. Rozhdestvenskii, *Trans. State Inst. Applied Chem. (U.S.S.R.)*, 20, 47 (1934); through *Chem. Abstracts*, 29, 2311 (1935).

⁴E. Baudrimont, *Ann. chim. phys.*, [4] 2, 12 (1864).

⁵C. A. Jacobson, *Encyclopedia of Chemical Reactions*, Vol. I, Reinhold Publishing Corp., New York, 1946, p. 205.

⁶M. J. Riban, *Compt. rend.*, 95, 1160 (1882); *Bull. soc. chim.*, [2] 39, 14 (1883).

⁷H. McCombie, B. C. Saunders and G. J. Stacey, *J. Chem. Soc.*, 1945, 380.

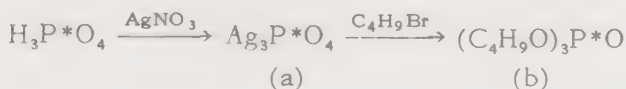
⁸B. C. Saunders and G. J. Stacey, *ibid.*, 1948, 695.

⁹T. P. Dawson, TDMR 832, Diisopropyl Fluorophosphate. Available from Office of Technical Services, Dept. of Commerce, Washington 25, D. C.

¹⁰L. C. Craig, *Ind. Eng. Chem., Anal. Ed.*, 9, 441 (1937).

¹¹B. C. Saunders and T. S. Worthy, *J. Chem. Soc.*, 1950, 1320.

BUTYL PHOSPHATE-P³²



W. H. Baldwin and C. E. Higgins, *J. Am. Chem. Soc.*, 74, 2431 (1952).

A. Procedure

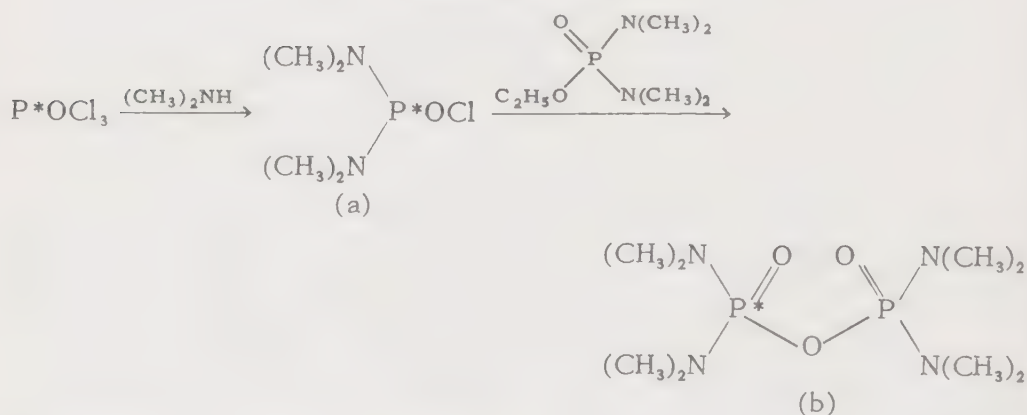
(a) *Silver Phosphate-P³²*. In a 400-ml. three-necked flask, fitted with an efficient stirrer, is placed a solution of 56.1 g. (0.33 mole) of silver nitrate in 50 ml. of water. A phosphoric acid solution, prepared from 68.6 mc. of phosphoric-P³² acid and 0.1 mole of reagent grade phosphoric acid in a volumetric flask (Note 1), is added. Then, with stirring, 3 *N* ammonium hydroxide is added dropwise, at room temperature, until the pH of the supernatant liquid is 6-7 and remains in this range for 15 minutes (Note 2). With stirring, the precipitate is allowed to digest at room temperature for 16 hours. The mother liquor is removed with a filter stick, and the precipitate is washed with 1.5 l. of water added in 10 equal portions. After the product is dried in an oven at 110° for 18 hours, it is broken up and returned to the oven for 1 1/2 hours. The dry yellow powder weighs 42.3 g. (100%) (Note 3).

(b) *Butyl Phosphate-P³²*. To the 42.3 g. of silver phosphate-P³², in a flask equipped with a stirrer and reflux condenser, is added 83 g. (0.6 mole) of butyl bromide (Note 4). The reaction mixture is refluxed for 8 hours, cooled and then extracted with three 50-ml. portions of ether. The residual cake is heated at 110° for 2 hours, broken up with a stirring rod and refluxed with 83 g. of butyl bromide for 8 hours. The reaction mixture is again extracted with three 50-ml. portions of ether which are combined with those from the first treatment. The ether solution is washed, first with an equal volume of 0.1 *N* hydrochloric acid, then a like amount of 0.1 *N* sodium hydroxide, twice with an equal volume of distilled water, and finally is dried over anhydrous potassium carbonate. After the ether and unreacted butyl bromide are removed by distillation, 14.6-16 g. (55-60%) of product, b.p. 157° (16 mm.), is collected.

B. Notes

1. The volume was adjusted with distilled water, and 0.05-ml. aliquots were removed for P^{32} assay.
2. Wide-range pH test paper was satisfactory for the adjustment, and approximately 100 ml. of 3 *N* ammonium hydroxide was required.
3. The combined filtrate and washings retained 0.5% of the total starting phosphorus- P^{32} .
4. The excess bromide serves as solvent during the reaction.

**TETRAMETHYLPHOSPHORODIAMIDIC
TETRAMETHYLPHOSPHORODIAMIDIC- P^{32}
ANHYDRIDE**



J. E. Gardiner and B. A. Kilby, J. Chem. Soc., 1950, 1769.

A. Procedure

(a) *Tetramethylphosphorodiamidic- P^{32} Chloride* (Note 1). The all-glass apparatus consists essentially of two parts (Figure XIX, 4): a test tube-like reaction vessel attached to a manifold, and a small distillation apparatus with sealed-on column and condenser. Reaching nearly to the bottom of the reaction tube is a center tube terminating in a fritted-glass disc (Note 2). The reaction tube, containing 0.93 g. (6 mmoles) of phosphoryl- P^{32} chloride and 10 ml. of ether, is attached to the apparatus. This solution is stirred with a stream of nitrogen and cooled in a Dry Ice-alcohol bath. With continued stirring (Note 3), a solution of 1.08 g. (24 mmoles) of dimethylamine in 10 ml. of ether is added slowly from a dropping funnel. The funnel is washed with 10 ml. of ether, and the solution is then allowed to warm to room temperature and left overnight with gas stirring. The reaction mixture is again cooled (Note 4) and filtered into the distillation apparatus. The solid residue of amine hydrochloride is washed 3 times with cold ether. After removal of the residual ether, 7 g. of

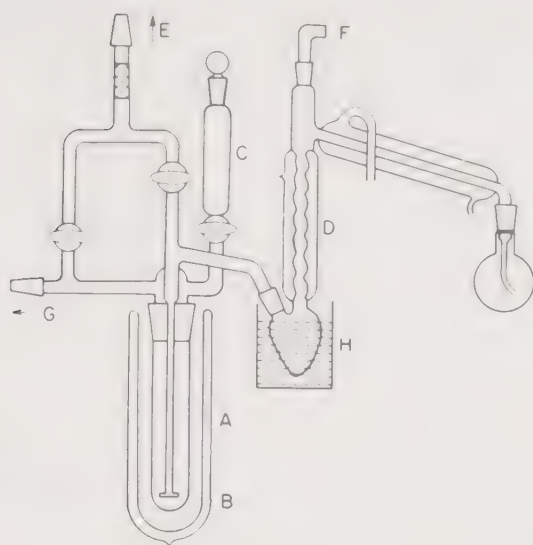


Fig. XIX, 4 Apparatus for the preparation of tetramethylphosphorodiamidic- P^{32} chloride (J. E. Gardiner and B. A. Kilby). A, reaction tube; B, fritted disk; C, dropping funnel; D, distillation apparatus with attached column and condenser; E, connection to dry nitrogen supply; F, connection to bubbler or vacuum line; G, connection to cold traps.

ordinary tetramethylphosphorodiamidic chloride is added in two portions. After each addition the diluted product is distilled at reduced pressure. The yield of product is 7.2 g., b.p. $62-64^{\circ}$ (1-1.1 mm.) (oil-bath temperature $82-84^{\circ}$).

(b) *Tetramethylphosphorodiamidic Tetramethylphosphorodiamidic- P^{32} Anhydride.* Tetramethylphosphorodiamidic- P^{32} chloride, 7.2 g., is dissolved in 15 ml. of dry benzene in a 50-ml. flask. To this solution is added 10 g. of ethyl tetramethylphosphorodiamidate, and the mixture is heated overnight under reflux in a stirred oil-bath at $130-145^{\circ}$. Moisture is excluded with a calcium chloride tube. The mixture is then distilled through a short Vigreux column. A small first fraction, distilling up to 128° (1.5 mm.), is mainly unchanged ester. The yield of anhydride, b.p. $139-140^{\circ}$ (1.5 mm.) (Note 5), is 9.44 g. (78%).

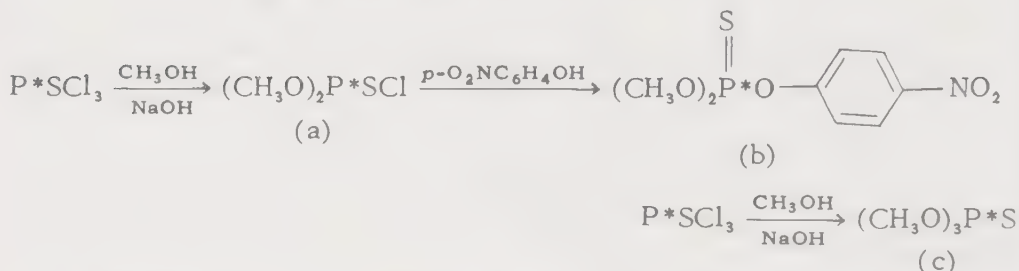
B. Notes

1. This compound is prepared according to an adaptation of the method of Cook, *et al.*¹
2. The tube serves either to stir the reactants with an inert gas or to filter the reaction mixture in transferring it into the distillation apparatus.
3. The effluent nitrogen stream is passed through traps.
4. Cooling reduces the solubility of dimethylamine hydrochloride in the ether solution.

5. The bath temperature was 160 °.

¹H. G. Cook, J. D. Ilett, B. C. Saunders, G. J. Stacey, H. G. Watson, I. G. E. Wilding and S. J. Woodcock, J. Chem. Soc., 1949, 2921.

METHYL PHOSPHOROTHIONATE-P³²



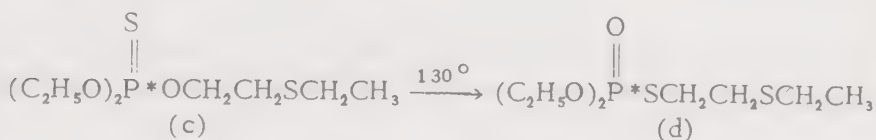
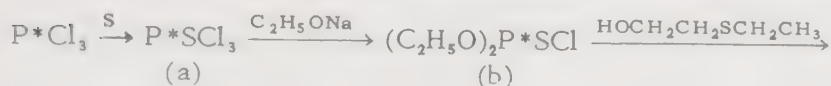
Ya. A. Mandel'baum, V. I. Lomakina and N. N. Mel'nikov, Doklady Akad. Nauk S.S.S.R. 96, 1173 (1954); through Chem. Abstracts, 49, 8843 (1955).

Procedure

(a) *Methyl Phosphorochloridothionate-P³²*. To 6.4 g. of thiophosphoryl-P³² chloride at -5 ° is added with stirring 14.9 ml. of a 5.6 M solution of sodium hydroxide in 60% methanol. Then after 1.5 hours at 20 °, the mixture is diluted with water, and the lower layer is separated, dried and distilled. The yield of methyl phosphorochloridothionate-P³², b.p. 66 ° (16 mm.), d_4^{20} 1.3350, n_D^{20} 1.4820 is 67%.

(b) *Dimethyl 4-Nitrophenyl Phosphorothionate-P³²*. A mixture of 2.3 g. of dimethyl phosphorochloridothionate-P³², 2 g. of 4-nitrophenol and 1.5 g. of powdered sodium carbonate in 7 ml. of acetone is refluxed with stirring for 3-4 hours. The mixture is filtered, and, after the filtrate is concentrated, it is washed with water, dried, and evaporated to obtain dimethyl 4-nitrophenyl phosphorothionate-P³², m.p. 35-36 °, in 65% yield.

(c) *Methyl Phosphorothionate-P³²*. To 3 g. of thiophosphoryl-P³² chloride at -5 ° is added with stirring 10.9 ml. of 5.6 M solution of sodium hydroxide in 60% methanol. After 1 hour at 20 °, the mixture is diluted with water, and the product is separated, dried and distilled. There is obtained a 72.5% yield of methyl phosphorothionate-P³², b.p. 73 ° (13 mm.), d_4^{20} 1.2190, n_D^{20} 1.4590.

DIETHYL S-[2-(ETHYLTHIO)ETHYL] PHOSPHOROTHIOLATE-P³²

T. R. Fukuto and R. L. Metcalf, *J. Am. Chem. Soc.*, **76**, 5103 (1954).

A. Procedure

(a) *Thiophosphoryl-P³² Chloride*. According to the method of Knötz¹ (Note 1), a mixture of 6.5 g. of phosphorus-P³² trichloride, 1.8 g. of sulfur and 0.18 g. of anhydrous aluminum chloride is heated on a water-bath, under reflux, for 10 minutes. The reaction mixture is cooled and treated with a large volume of water in a separatory funnel. The thiophosphoryl-P³² chloride, which settles to the bottom, is separated and dried over calcium chloride. Distillation of the crude product yields 5.0 g. (62%) of thiophosphoryl-P³² chloride, b.p. 60–63° (90 mm.).

(b) *Ethyl Phosphorochloridithionate-P³²*. To 5.0 g. of thiophosphoryl-P³² chloride, stirred mechanically and cooled in an ice-salt bath, is added slowly a solution of 1.39 g. of sodium in 25 ml. of absolute alcohol (Note 2). The mixture is poured into 75 ml. of ice-water and extracted twice with methylene chloride. After the methylene chloride solution is dried over calcium chloride, the solvent is removed and the product is distilled. The yield of ethyl phosphorochloridithionate-P³², b.p. 74° (15 mm.), is 5.0 g. (90%).

(c) *Diethyl 2-(Ethylthio)ethyl Phosphorothionate-P³²* (Note 2). A mixture of 5.0 g. of ethyl phosphorochloridothionate-P³², 2.81 g. of ethyl-2-hydroxyethyl sulfide,² 5.3 g. of anhydrous potassium carbonate, 0.106 g. of copper powder and 5 ml. of benzene is heated at 60° (Note 3). The yield is 6.0 g. (88%).

(d) *Diethyl S-[2-(Ethylthio)ethyl] Phosphorothiolate-P³²*. To effect isomerization, 93 mg. of the thiono isomer is heated in an open test-tube at 120–130° for 4 hours (Note 4).

B. Notes

1. Thiophosphoryl chloride was obtained¹ in 97% yield by the direct combination of sulfur and phosphorus trichloride. The reaction was catalyzed with anhydrous aluminum chloride and was complete within 10 minutes at the boiling point of phosphorus trichloride.

2. The procedure is according to that described by Schrader,³ and by Gardner and Heath.⁴

3. The product was not distilled. Chromatographic analysis showed that the radioactive constituent was 96.5% thiono isomer, (b). The reported b.p.⁴ of this compound is 92° (0.5 mm.).

4. Chromatographic analysis showed that complete isomerization of the thiono, (c), to the thiol isomer, (d), had occurred. A kinetic study of the isomerization showed it to be a first order reaction and, of several solvents tried, ethyl alcohol and chloroform apparently accelerated the reaction at a given temperature. Gardner and Heath⁴ reported the boiling point of diethyl S-[2-(ethylthio)ethyl] phosphorothiolate as 107-110° (0.5 mm.). They also synthesized the two isomers independently and demonstrated that the commercial insecticide, "Systox," is a mixture of the two.

C. Other Preparations

Diethyl S-[2-(ethylthio)ethyl] phosphorothiolate-P³² has been prepared⁴ in 30.3% yield (based on phosphorus-P³² trichloride) from sodium 2-(ethylthio)ethylmercaptide⁵ and ethyl phosphorochloridate-P³².

Diethyl 2-(ethylthio)ethyl phosphorothionate-P³² has been prepared⁴ in 60% yield by a procedure similar to that described.

Thiophosphoryl-P³² chloride has been prepared in 53% yield by the reaction of phosphorus-P³² trichloride and sulfur in a sealed tube at 150° for 2 hours.⁶

Thiophosphoryl-P³² chloride has also been prepared^{7,8} in 62.7% yield by the reaction of phosphorus pentasulfide and phosphorus-P³² pentachloride at 150° for 2 hours. The latter compound was prepared in quantitative yield by the reaction of red phosphorus-P³² and dry chlorine during 4 hours.

¹F. Knötz, *Österr. Chem.-Ztg.*, 50, 128 (1949); through Chem. Abstracts, 43, 9394 (1949).

²W. Steinkopf, J. Herold and J. Stohr, *Ber.*, 53, 1007 (1920).

³G. Schrader, *Die Entwicklung neuer Insektizide auf Grundlage organischer Fluor und Phosphor-Verbindungen*, Monograph No. 62, *Angewandte Chemie*, 1952.

⁴K. Gardner and D. F. Heath, *Anal. Chem.*, 25, 1849 (1953).

⁵R. L. Frank and P. V. Smith, *J. Am. Chem. Soc.*, 68, 2103 (1946).

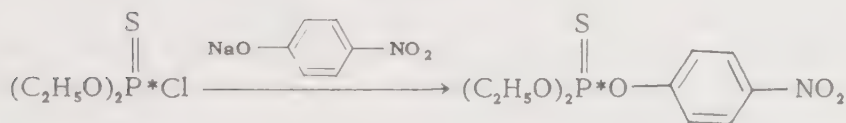
⁶D. H. Murray and J. W. T. Spinks, *Can. J. Chem.*, 30, 497 (1952).

⁷S. Lockau, M. Lüdicke and F. Weygand, *Naturwissenschaften*, 38, 350 (1950).

⁸S. Lockau and M. Lüdicke, *Z. Naturforsch.*, 7b, 389 (1952).

DIETHYL 4-NITROPHENYL PHOSPHOROTHIONATE-P³²
(Parathion-P³²)

METHOD I



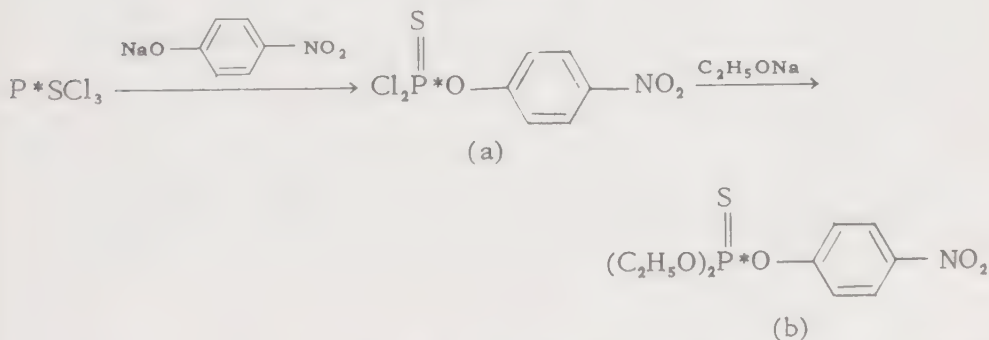
C. H. Murray and J. W. T. Spinks, *Can. J. Chem.*, 30, 497 (1952).

A. Procedure

Parathion-P³² is prepared according to a modification of the following procedure of Fletcher, *et al.*¹ (Note 1).

A mixture of 37.7 g. of ethyl phosphorochloridothionate-P³², 32.2 g. of sodium 4-nitrophenoxide and 200 ml. of ethanol (Note 2) is refluxed for 1 hour. After the mixture is cooled to 20° and filtered, the filtrate is concentrated *in vacuo*. The residue is heated for a short time in an oil-bath at 100–110°, at 0.5 mm. (Note 3). The crude product, dissolved in 100 ml. of toluene, is washed with 5% sodium carbonate solution and then with water. After the solution is dried over Drierite, the solvent is removed under reduced pressure; the yield is 43.5 g. (75%). Parathion is a yellow oil, b.p. 157–162° (6 mm.), n_D^{25} 1.5370.

METHOD II



S. Lockau, M. Lüdicke and F. Weygand, *Naturwiss.*, 38, 350 (1951); S. Lockau and M. Lüdicke, *Z. Naturforsch.*, 7b, 389 (1952).

A. Procedure

(a) *4-Nitrophenyl Phosphorochloridothionate-P³²*. To 16.3 g. of pure thiophosphoryl-P³² chloride in a 40-ml. flask is slowly added, in small portions, 3.1 g. of sodium 4-nitrophenoxide with shaking and cooling (Note 4). The mixture is heated under reflux on a water-bath with shak-

ing. After a short time the orange color of the sodium salt changes to yellow-brown and then brown; the reaction is complete in 3 hours. The reaction mixture is cooled and thoroughly mixed with 20 ml. of dry benzene. The precipitated salt is collected on a dry Büchner funnel and washed 3 times with small portions of dry benzene (Note 5). The filtrate is concentrated under vacuum (Note 6) on a water-bath; the distillate contains the excess thiophosphoryl- P^{32} chloride. The product is a dark brown viscous oil which solidifies at room temperature; yield 1.86 g. (7.1%).

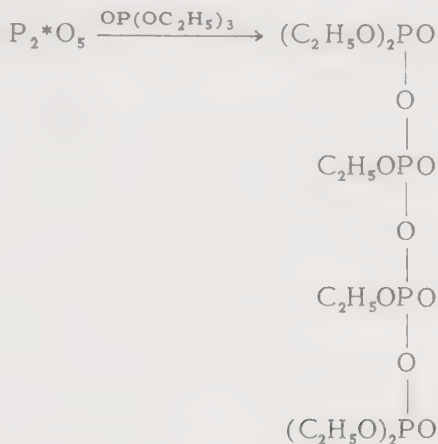
(b) *Diethyl 4-Nitrophenyl Phosphorothionate- P^{32}* , (*Parathion- P^{32}*). To the 1.86 g. of intermediate is added dropwise 2 g. of sodium ethylate in ethanol (15% solution), with cooling and shaking of the mixture. Then, with occasional shaking the mixture is heated for 2.5 hours under reflux on a boiling water-bath. After the mixture is cooled, the excess sodium ethylate is destroyed by passing a stream of carbon dioxide through the solution for 20 minutes. The precipitated salt is collected on a dry Büchner funnel, and the clear reddish-brown filtrate is concentrated under vacuum (<20 mm.) on a water-bath. The product is a viscous dark-brown oil; the yield is 0.840 g. (42%).

B. Notes

1. Very few experimental details were given by Murray and Spinks.
2. An experiment in which the reaction was run at 125° in chlorobenzene² gave a 79% yield of parathion after 51 hours.
3. Unreacted ethyl phosphorochloridothionate- P^{32} is recovered in this manner.
4. The sodium 4-nitrophenoxide must be dry and free of ethanol.
5. The salt remains reddish-brown in color.
6. The pressure should not exceed 20 mm.

¹J. H. Fletcher, J. C. Hamilton, I. Hechenbleikner, E. I. Hoegberg, B. J. Serfl and J. T. Cassaday, J. Am. Chem. Soc., 70, 3943 (1948).

²J. T. Thurston, FIAT Final Report No. 949, October 14, 1946 (PB-60890).

HEXAETHYL P_2^{32} -TETRAPOLYPHOSPHATE

R. W. Brauer and R. L. Pessotti, *Science*, 110, 395 (1949); R. W. Brauer, *J. Pharmacol., Exptl. Therap.*, 92, 162 (1948).

Procedure

(a) *Bis(phosphoric- P^{32}) Anhydride*. Isotopic phosphorus pentoxide might be prepared by the reaction of silver phosphate- P^{32} with acetyl chloride,¹ by the reaction of phosphoryl- P^{32} chloride with potassium chlorate² or by the reaction of phosphoryl- P^{32} chloride with silver acetate.¹

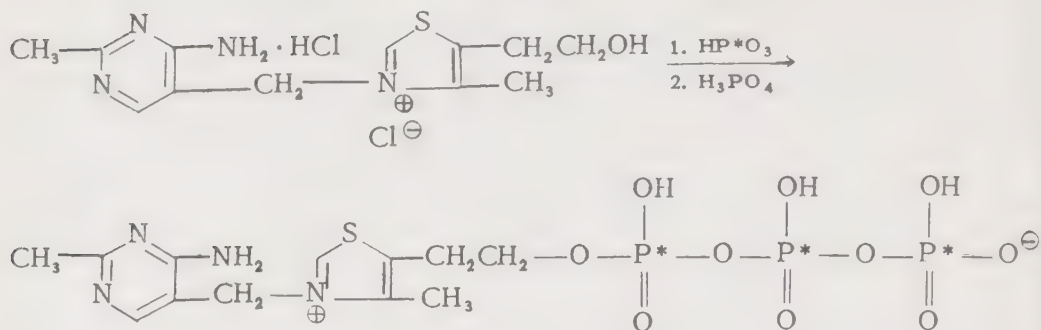
(b) *Hexaethyl P_2^{32} -Tetrapolyphosphate*. This compound is prepared by the reaction of bis(phosphoric- P^{32}) anhydride with ethyl phosphate, in a molar ratio of 1:2, at 55° for 120 minutes and then at 100° for another 60 minutes. According to Woodstock^{3,4} hexaethyl tetrapolyphosphate has the following physical properties: d_{25}^{25} 1.280, n_D^{25} 1.425 and decomposition point 145–150°.

¹A. Bechamp and C. Saintpierre, *Compt. rend.*, 55, 59 (1862).

²G. Oddo, *Gazz. chim. ital.*, 29, 2, 333 (1899).

³W. H. Woodstock, U. S. 2,402,703; through *Chem. Abstracts*, 40, 5444 (1946).

⁴H. Adler and W. H. Woodstock, *Chem. Industries*, 51, 516 (1942).

THIAMINE TRIPHOSPHATE- P_3^{32} 

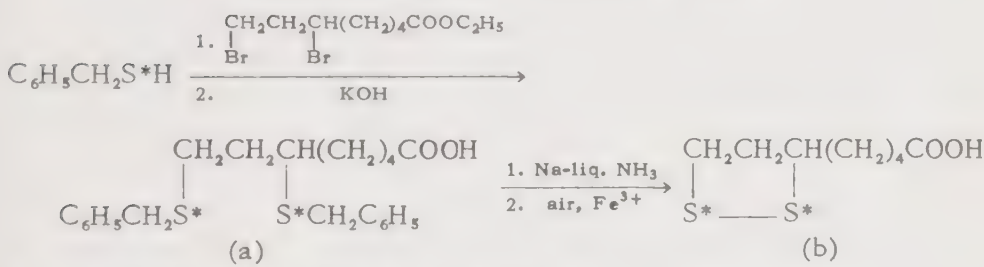
M. Koike, *Vitamins (Japan)*, 6, 420 (1953); through *Chem. Abstracts*, 48, 5194 (1956).

Procedure

A mixture of 0.2 g. of thiamine hydrochloride and 4.4 ml. of aqueous metaphosphoric- P_3^{32} acid is heated at 105° for 15 minutes. The mixture is cooled and diluted with 15 ml. of water containing 0.1 ml. of 85% phosphoric acid, and the insoluble material is removed by centrifugation. The addition of 50 ml. of acetone-alcohol (1:1) to the supernatant liquid precipitates the ester.

SULFUR-35 COMPOUNDS

α -LIPOIC-S₂³⁵ ACID
(2,3-Dithiacyclopentanevaleric-S₂³⁵ Acid)



R. C. Thomas and L. J. Reed, J. Am. Chem. Soc., 77, 5446 (1955).

A. Procedure

(a) *6,8-Bis(benzylthio)octanoic-S₂³⁵ Acid.* To a mixture of 273.2 mg. (2.2 mmoles) of α -toluenethiol-S³⁵ and 330 mg. (1 mmole) (Note 1) of ethyl 6,8-dibromoöctanoate,¹ in a 5-ml. flask, is added a solution of 2.2 mmoles of sodium ethoxide in 2 ml. of absolute ethanol. The mixture, stirred by means of a magnetic stirrer, is heated under reflux in an atmosphere of nitrogen for 5 hours. At this time, the reaction mixture is cooled to room temperature, and 132 mg. (2 mmoles) of potassium hydroxide is added. The flask is stoppered, and stirring is continued for 20 hours at room temperature. The reaction mixture is poured into 10 ml. of water, and the resulting solution is extracted with two 2-ml. portions of peroxide-free ether. The aqueous phase is acidified with 6 N hydrochloric acid, and the product is extracted with five 2-ml. portions of peroxide-free ether. The combined ether extracts are washed successively with 2 ml. of water, two 1-ml. portions of ice-cold 5% sodium bicarbonate solution (Note 2) and 2 ml. of water. After the ether solution is dried over anhydrous sodium sulfate, the solvent is evaporated with a stream of nitrogen and finally under vacuum. The solid residue is dissolved in 1 ml. of warm benzene, and 4 ml. of hot Skellysolve B (Note 3)

is added gradually. The clear solution is seeded with a small crystal of product and then kept at room temperature overnight and finally in a refrigerator for 4 hours. The mother liquor is removed with a pipet, and the crystals are washed with three 2-ml. portions of cold Skellysolve B. The yield of 6,8-bis(benzylthio)octanoic- S_2^{35} acid, m.p. 68.5–69.5° (uncor.) (Note 4), is 304 mg. (71%).

(b) α -Lipoic- S_2^{35} Acid, (2,3-Dithiacyclopentanevaleric- S_2^{35} Acid). Into a 15 × 180-mm. tube, equipped with a Hershberg-type stirrer and immersed in a Dry Ice-isopropyl alcohol mixture, are added in succession 5 ml. of anhydrous liquid ammonia, 16 mg. of sodium wire and a solution of 50 mg. (0.13 mmole) of 6,8-bis(benzylthio)octanoic- S_2^{35} acid in 2 ml. of peroxide-free, anhydrous ether. The mixture is stirred until decolorized, and small pieces of sodium are then added until a permanent blue color results. After the blue color is discharged with ammonium chloride, the cooling bath is removed, and the liquid ammonia and ether are evaporated in a slow stream of nitrogen. To the residue is added 4 ml. of water, and the pH is adjusted to 9 with 2 N hydrochloric acid. To this solution are added 2 drops of 1% ferric chloride solution, and a stream of air is bubbled through the solution until the reddish color changes to pale yellow (approximately 15 minutes). The mixture is extracted with two 2-ml. portions of peroxide-free ether to remove some suspended solid material and is then acidified with 6 N hydrochloric acid. The product is extracted into three 1-ml. portions of chloroform, which are combined, dried over sodium sulfate and then evaporated to dryness in a small sublimation apparatus with a stream of nitrogen. The last traces of solvent are removed at 10^{-4} mm. The crude product is sublimed at 10^{-4} mm. and 90° onto a surface cooled with Dry Ice (Note 5). The solid product is washed from the cold finger with dry benzene, which is evaporated with a slow stream of nitrogen and finally *in vacuo*. The yield of microcrystalline product is 19.5–22.8 mg. (73–86%), m.p. 60.5–61.5° (uncor.) (Note 4), ϵ max. 147 (332 m μ).

B. Notes

1. In preliminary experiments, it was found that use of a 10% excess of benzyl mercaptan resulted in a higher yield of crystalline 6,8-bis(benzylthio)octanoic acid, based on mercaptan, than did the use of the theoretical amount of mercaptan or a 10% excess of the dibromo ester.

2. In trial runs, it was found that the extraction with sodium bicarbonate solution removed unidentified impurities which otherwise interfered with crystallization of the 6,8-bis(benzylthio)octanoic acid, which is only very sparingly soluble in 5% sodium bicarbonate solution.

3. A hexane fraction, b.p. 60–68°.

4. Melting points were determined on a hot stage viewed with a microscope equipped with polaroid discs.

5. In preliminary experiments, attempts to crystallize the crude product from Skellysolve B produced varying amounts of insoluble, viscous material and, consequently, low yields of crystalline product. When the crude product was sublimed only traces of residue remained.

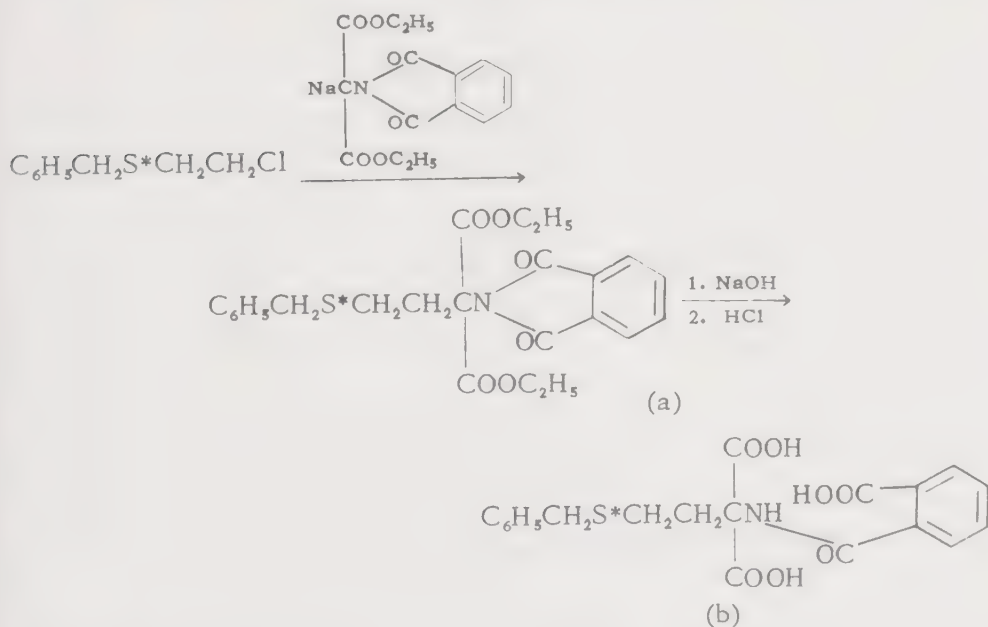
C. Other Preparations

6,8-Bis(benzylthio)octanoic- S^{35} acid has been prepared² essentially according to the procedure described and the method of Reed and Niu.¹ α -Lipoic- S^{35} acid has also been prepared² at two radioactivity levels and the radiation-induced decomposition was studied.

¹L. J. Reed and Ching-I Niu, J. Am. Chem. Soc., 77, 416 (1955).

²P. T. Adams, *ibid.*, 77, 5357 (1955).

[2-(BENZYLTHIO)ETHYL](2-CARBOXYBENZAMIDO)-MALONIC- S^{35} ACID



H. Tarver and C. L. A. Schmidt, J. Biol. Chem., 130, 67 (1939).

A. Procedure

(a) Ethyl α -[2-(benzylthio)ethyl]-1,3-dioxo-2-isoindolinemalonate- S^{35} , [Ethyl 2-(benzylthio)ethylphthalimidomalonate- S^{35}]. A mixture of 4.4 g. of benzyl 2-chloroethyl sulfide- S^{35} and 8 g. of ethyl 1,3-dioxo- α -sodium-2-isoindolinemalonate (Note 1) is heated in a stoppered flask at 170° for 4.5 hours. The reaction mixture is extracted several times with toluene; removal of the solvent leaves the product as an oil (Note 2).

(b) [2-(Benzylthio)ethyl](2-carboxybenzamido)malonic-S³⁵ Acid. The above oily product is heated on a steam-bath with 10 ml. of 95% alcohol and 25 ml. of 5 *N* sodium hydroxide. Hydrolysis is complete in 2 hours, and the cold hydrolysate is treated with 125 ml. of ice-cold 1 *N* hydrochloric acid. After the solution is refrigerated for 30 minutes, 40 ml. of 12 *N* hydrochloric acid is added. The product crystallizes slowly from the refrigerated solution. It is collected after 2 days and washed with a few ml. of ice-cold water and dried (Note 3).

B. Notes

1. The ethyl 1,3-dioxo- α -sodium-2-isoindolinemalonate is freshly prepared.

2. In preliminary experiments, it did not appear possible to crystallize this product.

3. A sample of thio acid, purified by dissolution in dilute alkali and reprecipitation with hydrochloric acid, melted at 110-111° (cor.) with decomposition.

C. Other Preparations

Ethyl α -[2-(benzylthio)ethyl]-1,3-dioxo-2-isoindolinemalonate-S³⁵ has been prepared¹ from benzyl 2-chloroethyl sulfide-S³⁵ by a procedure similar to that described.

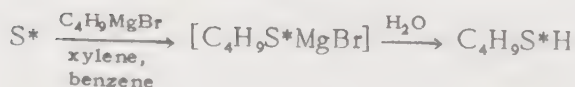
Ethyl α -[2-(benzylthio)ethyl]-1,3-dioxo-2-isoindolinemalonate-S³⁴ has been prepared² from benzyl 2-chloroethyl sulfide-S³⁴, also by a similar procedure.

¹A. M. Seligman, A. M. Rutenburg and H. Banks, *J. Clin. Invest.*, 22, 275 (1943).

²G. W. Kilmer and V. du Vigneaud, *J. Biol. Chem.*, 154, 247 (1944).

1-BUTANETHIOL-S³⁵ (Butyl Mercaptan-S³⁵)

METHOD I



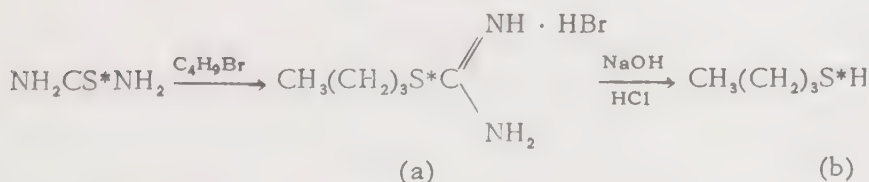
J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, *J. Am. Chem. Soc.*, 70, 2547 (1948).

A. Procedure

To a cold solution of sulfur-S³⁵ in 5 ml. of dry xylene, contained in a 50-ml. centrifuge cone, is added 40 ml. of 6 *N* butylmagnesium bromide in

benzene. The solutions are mixed and after 3-4 hours, the tube is filled with petroleum ether (b.p. 50-60°), and the mixture is centrifuged. The liquid is decanted into a 100-ml. centrifuge tube. The residue of (butylthio)magnesium-S³⁵ bromide is stirred with 25 ml. of petroleum ether and centrifuged. The wash liquid is added to the first mother liquor in the 100-ml. tube; the volume is increased to 100 ml. with petroleum ether, whereupon an additional amount of precipitate is obtained, which is added to the main residue. The combined precipitates are washed with 25 ml. of petroleum ether by centrifugation. The Grignard reagent is then suspended in a few ml. of petroleum ether under an atmosphere of nitrogen, cooled in an ice-bath, and treated with 2 ml. of 5 N hydrochloric acid. The mixture is stirred until the magnesium salts dissolve. The petroleum ether layer is separated and washed with 2 ml. of water. The combined aqueous solutions are extracted with small portions of petroleum ether until a test (Note 1) for the sulfhydryl group is negative. All of the petroleum ether solutions containing 1-butanethiol-S³⁵ are combined (Note 2).

METHOD II



C. Walling, J. Am. Chem. Soc., 70, 2561 (1948).

A. Procedure

(a) *S*-Butylisothiuronium-S³⁵ Bromide. In a 200-ml. flask equipped with a dropping funnel and condenser (Note 1) are placed 10 ml. of thiourea-S³⁵ solution (about 2 mmoles), 0.76 g. (10 mmoles) of inactive thiourea and 3 ml. of butyl bromide. The mixture is refluxed 2½ hours under a hydrogen atmosphere. After the mixture is cooled, the volatile material is distilled into a liquid nitrogen cooled trap under vacuum (Note 2).

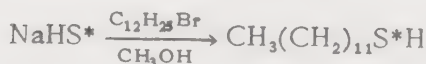
(b) *1*-Butanethiol-S³⁵, (*Butyl Mercaptan*-S³⁵). With the condenser in the reflux position, hydrogen is readmitted, and 10 ml. of *N* sodium hydroxide is added through the funnel. The solution is then refluxed for 45 minutes to hydrolyze the *S*-butylisothiurea-S³⁵. Then the apparatus is arranged for distillation, 10 ml. of 0.5 *N* hydrochloric acid is added, and approximately half of the contents of the flask is distilled into 10 ml. of chlorobenzene in a separatory funnel (Note 3). After the receiver is shaken gently, the solvent is separated, and the aqueous layer is extracted with a total of 15 ml. of chlorobenzene. The combined chlorobenzene solution contains approximately 0.4 mmole of product (Note 4).

B. Notes

1. The nitroprusside test¹ was used.
2. In this instance, the product was used in a subsequent reaction without isolation. 1-Butanethiol has the following physical properties: m.p. -115.9° ; b.p. 98.2° (766 mm.); d_4^{20} 0.8337; n_D^{20} 1.44402; slightly soluble in water and very soluble in alcohol and ether.
3. The condenser could be swiveled for either reflux or distillation.
4. The *S*-butylisothiuronium- S^{35} bromide was not isolated.
5. Chlorobenzene was chosen as an inert solvent with density greater than water.
6. The chlorobenzene solution was used directly without isolation of the product. The preliminary experiments indicated the yield to be nearly quantitative. Since 1-butanethiol boils at 98° and chlorobenzene at 132° , it might be advantageous to use a low-boiling solvent, such as ether, when the preparation is on the millimole scale and the product is to be isolated.

¹I. W. Grote, J. Biol. Chem., 93, 25 (1931).

DODECANETHIOL- S^{35}
(Dodecyl Mercaptan- S^{35})



W. E. Mochel and J. H. Peterson, J. Am. Chem. Soc., 71, 1426 (1949).

Procedure

A methanol solution containing sodium hydrosulfide- S^{35} and dodecyl bromide is heated in a pressure tube for 3 hours at 120° . After removal of methanol, the dodecyl mercaptan- S^{35} , b.p. $104-110^{\circ}$ (3 mm.), is purified by distillation.

α -TOLUENETHIOL- S^{35}
(Benzyl Mercaptan- S^{35})

METHOD I

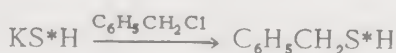


R. C. Thomas and L. J. Reed, J. Am. Chem. Soc., 77, 5446 (1955).

A. Procedure (Note 1)

To a solution of 0.65 mmole of sulfur-S³⁵ in 5 ml. of benzene, contained in a 40-ml. centrifuge tube, is added 5 ml. of a 0.5 M solution of benzylmagnesium bromide in benzene, under an atmosphere of dry nitrogen. The tube is stoppered and left at room temperature for 12-15 hours. The resulting suspension is centrifuged, and the excess Grignard solution is withdrawn with a pipet. The solid is first washed, by centrifugation, with three 5-ml. portions of Skellysolve B (Note 2) and then suspended in 5 ml. of ice-cold Skellysolve B. To the suspension is added 1 ml. of 6 N hydrochloric acid, and the mixture is stirred until free of solids. The aqueous layer is removed, and the organic layer is washed with 1-ml. portions of water. The combined aqueous layers are extracted with 1-ml. portions of Skellysolve B until a negative nitroprusside test is obtained. Analysis of a small aliquot of the combined organic layers indicated the yield of α -toluenethiol-S³⁵ to be 78% (Note 3).

METHOD II



A. M. Seligman, A. M. Rutenburg and H. Banks, J. Clin. Invest., 22, 275 (1943).

Hydrogen sulfide-S³⁵, passed over a small amount of anhydrous calcium chloride, is absorbed in an equivalent of cold 2 N alcoholic potassium hydroxide (0.065 mole). An equivalent amount of benzyl chloride, dissolved in 2 volumes of dry ether, is added at once, and the mixture is cooled. The fluffy precipitate of potassium hydrosulfide-S³⁵ is gradually replaced by a granular precipitate of potassium chloride. The mixture is then heated to the boiling point, and the hydrogen sulfide-S³⁵, which is driven off, is absorbed in additional alcoholic potassium hydroxide (Note 4), treated with benzyl chloride, and added to the original reaction mixture, which is then set aside for several hours. The ether solution is filtered and distilled to obtain 6 g. (74%) of α -toluenethiol-S³⁵ and 1.5 g. (4.6%) of benzyl sulfide-S³⁵. Reduction of the latter compound with sodium and liquid ammonia gives 0.3 g. (4%) of α -toluenethiol-S³⁵, making the total 6.3 g. (78%).

B. Notes

1. This procedure is a modification of the method of Wood, *et al.*¹

2. This is a hexane fraction, b.p. 60-68°; petroleum ether, b.p. 35°, has also been used.¹

3. Analysis for sulphydryl groups by micro-titration with standard iodine solution was used.

4. Ten per cent of the initial amount of 2 N solution is used.

C. Other Preparations

α -Toluenethiol-S³⁵ has been prepared by the reaction of sulfur-S³⁵ with benzylmagnesium bromide¹ and in 85–88% yield² by the similar reaction with benzylmagnesium chloride, on a 5-mmole scale. The latter reaction employed at a 65-mmole level³ gave 80% of distilled α -toluenethiol-S³⁵, b.p. 194°. α -Toluenethiol-S³⁵ has been prepared in 70% yield by the reaction of potassium hydrosulfide-S³⁵ with benzyl chloride.⁴

α -Toluenethiol-S³⁴ has been prepared⁵ in 88% yield, on a macro scale, from sulfur-S³⁴ and benzylmagnesium bromide.

¹J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, *J. Am. Chem. Soc.*, **70**, 2547 (1948).

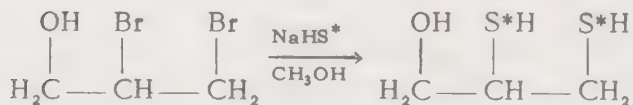
²P. T. Adams, *ibid.*, **77**, 5357 (1955).

³A. M. Seligman, A. M. Rutenburg and H. Banks, *J. Clin. Invest.*, **22**, 275 (1943).

⁴H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **130**, 67 (1939).

⁵G. W. Kilmer and V. du Vigneaud, *ibid.*, **154**, 247 (1944).

2,3-DIMERCAPTO-1-PROPANOL-S₂³⁵



R. A. Peters, G. H. Spray, L. A. Stochen, C. H. Collie, M. A. Grace and G. A. Wheatley, *Biochem. J.*, **41**, 370 (1947).

A. Procedure

A solution of sodium hydrosulfide-S³⁵ is prepared by passing hydrogen sulfide-S³⁵ in a stream of nitrogen into a solution of 150 mg. of sodium in 5 ml. of ethanol, cooled to -20°. To this solution is added a solution of 12 g. of ammonium hydrosulfide (Note 1) in 130 ml. of cold methanol. The resulting mixture is heated with 10.9 g. of 2,3-dibromo-1-propanol in a sealed glass bottle for 15 hours at 90–95°. Excess hydrogen sulfide-S³⁵ and the solvent are removed *in vacuo*; the residue is made acid to Congo red with 0.1 N hydrochloric acid, and the resulting solution is saturated with ammonium sulfate. The product is exhaustively extracted with pure benzene, and the extract is dried over sodium sulfate. The solvent is then removed *in vacuo*, and the residue is distilled at low pressure (Note 2). The yield is 3.5 g. (56%).

B. Notes

1. Ammonium hydrosulfide was prepared by passing dry hydrogen sulfide and dry ammonia into anhydrous ether at -10°.

2. According to Sjöberg,¹ the physical properties of 2,3-dimercapto-1-propanol are: b.p. 91.5–92° (1.7 mm.), 82–84° (0.8 mm.); d_{20} 1.2415; n_D^{20} 1.5741.

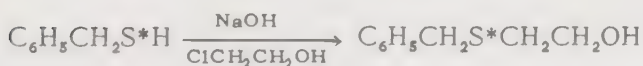
C. Other Preparations

2,3-Dimercapto-1-propanol-S³⁵ has been prepared by Young,² in 46% yield, using an adaptation of the method of Sjöberg¹ which employs only sodium hydrosulfide-S³⁵ in the reaction with 2,3-dibromo-1-propanol. The reaction was run in a sealed tube at 30° for 80 hours.

¹B. Sjöberg, *Ber.*, 75, 13 (1942).

²L. Young and S. D. Simpson, *Proc. XI Intern. Congr. Pure and Applied Chem. (London)*, 2, 537 (1947); *Chem. Abstracts*, 45, 5323 (1951).

2-(BENZYLTHIO)ETHANOL-S³⁵ (Benzyl 2-Hydroxyethyl Sulfide-S³⁵)

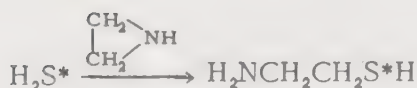


J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, *J. Am. Chem. Soc.*, 70, 2547 (1948).

Procedure

To the α -toluenethiol-S³⁵, prepared from 0.5 mmole of barium sulfate-S³⁵ and dissolved in a mixture of petroleum ether and ether, are added a solution of 36 mg. of ethylene chlorohydrin in 1 ml. of ether and 1 ml. of *N* sodium hydroxide solution. The two layers are thoroughly mixed by bubbling the alkali through the organic layer with the aid of a pipet. The mixture is then gently heated in a hot water-bath until the solvent evaporates. If the aqueous solution is not alkaline or not free of α -toluenethiol-S³⁵, additional alkali or ethylene chlorohydrin or both are added, and warming is continued until all the thiol-S³⁵ has reacted. Then, the 2-(benzylthio)ethanol-S³⁵ is extracted with one 5-ml., one 3-ml., and three 1-ml. portions of ether. The combined ether extracts, in a 15-ml. centrifuge tube, are washed with 1 ml. of water. The ether solution is heated in a water-bath to remove the solvent, and the last traces of ether are removed under vacuum.

2-AMINOETHANETHIOL-S³⁵
(Cysteamine-S³⁵)



C. Davison and R. Salvador, Dept. of Pharmacology, The George Washington Univ. School of Medicine, Washington, D. C., unpublished work.

A. Procedure (Note 1)

In 300 ml. of chilled absolute ethanol is dissolved 2 ml. of ethylenimine, 1.64 g. (0.038 mole) (Note 2), and the solution is flushed with nitrogen, leaving a nitrogen atmosphere in the reaction vessel (Note 3). Hydrogen sulfide-S³⁵, from 8.0 g. of barium sulfide-S³⁵, is passed into the alcoholic solution during one hour (Note 4). The alcoholic solution is then kept at room temperature for 24 hours with exclusion of air. A solution of 3.93 g. (0.029 mole) of salicylic acid, dissolved in a small volume of ethanol, is added to the reaction mixture, still under a nitrogen atmosphere. The solvent is then removed *in vacuo* at room temperature. The crystalline cysteamine-S³⁵ salicylate (Note 5) is recrystallized from ethanol, until free of excess salicylic acid. The yield of the salicylate salt, m.p. 103.5–104° (Note 6), is 3.7 g. (45% based on ethylenimine) (Note 7).

B. Notes

1. The procedure of Bogert and Mills¹ was modified to permit use of a limited amount of hydrogen sulfide-S³⁵.
2. Excess ethylenimine results in a by-product, 2,2'-thiobis(ethylamine).
3. It is very important that oxygen be excluded if the thiol, rather than the disulfide, is desired.
4. Unreacted hydrogen sulfide-S³⁵ is collected in a trap containing cadmium sulfate or cupric chloride solution.
5. Cysteamine-S³⁵ free base may be isolated directly but is very hygroscopic and is easily oxidized. The dry salicylate salt is stable and may be freed of salicylic acid by ether extraction of an acidified aqueous solution.
6. A sample obtained from Bacq, who originally described the salicylate salt,² melted at 103–103.8°.
7. Chemical and radiochemical purity was established in several ways. Paper chromatography in water-saturated phenol:ethyl formate:0.2 M citrate buffer at pH 5 (2:2:1 v/v) revealed radioactivity largely at the cysteamine spot (*R_f* 0.79) with a trace of the disulfide, cystamine

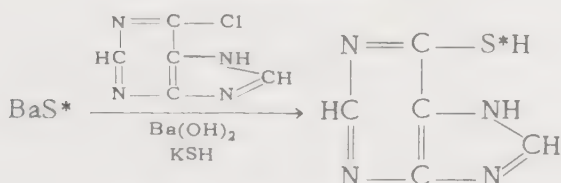
(R_f 0.42). Chromatography, on Dowex 50 resin columns, of the compound formed by reacting quantitatively the cysteamine with *N*-ethylmaleimide localized the activity chiefly in cysteamine (eluted with 0.1 M sodium carbonate), with small amounts present as cystamine (eluted with 0.1 M sodium hydroxide). Iodimetric titration of the sulfhydryl moiety showed the compound to be 96–98% pure.

¹M. T. Bogert and E. J. Mills, Jr., U.S. 2,358,786, Sept. 26, 1944.

²Z. M. Bacq, G. Dechamps, P. Fischer, A. Herve, H. LeBiham, J. Lecomte, M. Pirotte and P. Rayet, *Science*, 117, 633 (1953).

6-PURINETHIOL- S^{35}

(6-Mercaptopurine- S^{35})



G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, 76, 4027 (1954).

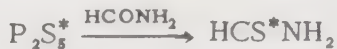
Procedure

To 2.5 g. (16.2 mmoles) of powdered 6-chloropurine in a glass bomb is added 10 ml. of water, 18 ml. of 2 *N* potassium hydrosulfide solution, and 1.9 ml. of 0.188 *N* barium hydroxide containing 1.729 mg. of barium sulfide- S^{35} . The bomb is sealed and heated in a boiling water-bath for 7 hours. A yellow, granular precipitate forms; after the tube is chilled it is opened, and 18 ml. of 2 *N* sodium hydroxide is added. Barium is precipitated by the addition of 2 g. of sodium sulfate. The solution is filtered, the precipitate is washed with water, and the combined filtrate and washings are placed in a 500-ml. flask connected to a series of three vessels, each containing 25 ml. of 0.5 *M* cadmium chloride solution. The last vessel is connected to a gas-drying cylinder containing sodium hydroxide pellets. Then, 37 ml. of 2 *N* hydrochloric acid is run slowly into the flask below the surface of the liquid, and a light yellow precipitate begins to form immediately. The liberated hydrogen sulfide- S^{35} is entrained in a stream of carbon dioxide for 3 hours and collected as cadmium sulfide- S^{35} . The precipitated product, 6-purinethiol- S^{35} , is collected, washed with water and dried in a vacuum desiccator; yield 2.28 g. (83%).

Additional product of lower activity is obtained by adding 1 g. of nonradioactive 6-purinethiol hydrate, dissolved in 4 ml. of 2 *N* sodium hydroxide, to the mother liquor. The solution is reacidified with 2 *N*

hydrochloric acid, and the yellow crystalline precipitate (0.87 g.) is collected, washed with water and dried.

THIOFORMAMIDE-S³⁵



Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, Doklady Akad. Nauk S. S. S. R., 91, 1129 (1953).

A. Procedure

With stirring, 4.5 g. of formamide, dissolved in 40-45 ml. of absolute ether, is added to 3.1 g. of phosphorus pentasulfide-S³⁵ during 20-30 minutes. The temperature of the reaction mixture is maintained at 20-22°, and, after it is stirred vigorously for 6-8 hours, the mixture is set aside for 20 hours at room temperature. Then, the ether solution is decanted from a viscous residue which is stirred for 10 minutes with 10-15 ml. more ether. After the mixture stands for a short time, the ether is again decanted and added to the first solution. Removal of ether under vacuum leaves 2.77 g. (65%) of thioformamide-S³⁵ (Note 1).

B. Notes

1. The product contained about 0.1 g. of elementary sulfur-S³⁵. The thioformamide-S³⁵ was dissolved in absolute alcohol and filtered. This solution was used immediately in the preparation of 5-(2-hydroxyethyl)-4-methylthiazole-S³⁵ in 17.6% yield. The latter compound was used in the synthesis of thiamin-S³⁵ (vitamin-B₁). Experimental details were not given for the preparation of these compounds.

THIOACETAMIDE-S³⁵



O. Nygaard, L. Eldjarn, and K. F. Nakken, Cancer Research, 14, 625 (1954).

A. Procedure

In the glass apparatus shown in Figure XX, 1, the barium sulfide-S³⁵ from 100 mg. of barium sulfate-S³⁵ is placed in the suspended inner cup.

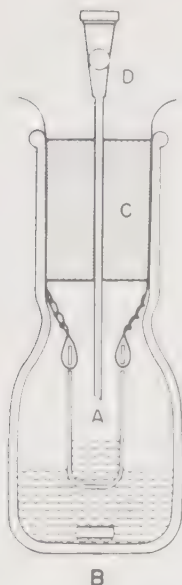


Fig. XX, 1 Apparatus for preparation of thioacetamide-S³⁵ (O. Nygaard, L. Eldjarn and K. F. Nakken). A, inner cup; B, magnetic stirrer; C, rubber stopper; D, hypodermic needle.

In the outer compartment of the apparatus are placed a solution of 200 mg. of thioacetamide in 9.5 ml. of dry ethanol (Note 1) and 0.5 ml. of 0.05 *N* sodium ethylate in dry ethanol. The apparatus is flushed with dry, oxygen-free nitrogen and stoppered, leaving a gas phase of approximately 20 ml. A hypodermic needle is then introduced through the greased rubber stopper, and the barium sulfide-S³⁵ is acidified with 1.5 ml. of 75% phosphoric acid by means of a syringe. The needle is carefully withdrawn to avoid loss of hydrogen sulfide-S³⁵. With magnetic stirring of the solution in the outer compartment, the reaction vessel is kept in darkness at room temperature for 4-7 days. After this period, two hypodermic needles are introduced through the stopper. Hydrogen chloride in absolute ethanol is introduced into the outer compartment in an amount sufficient to neutralize the sodium ethylate. Hydrogen sulfide-S³⁵ is flushed from the apparatus with nitrogen and trapped in sodium hydroxide. The solution of thioacetamide-S³⁵ is evaporated to dryness under reduced pressure and the product is recrystallized from benzene. The recovery of purified thioacetamide-S³⁵, m.p. 107.5-108.5°, is 50-60% (Note 2).

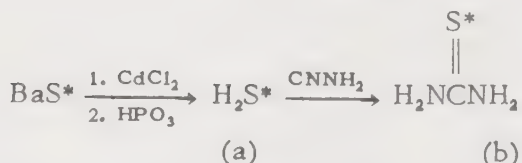
B. Notes

1. The "super dry" ethanol was prepared according to Lund and Bjerrum.¹

¹A. I. Vogel, *A Textbook of Practical Organic Chemistry*, 2nd. ed., Longmans, Green and Co., London, 1951, p. 166.

2. The compound gave but a single spot when chromatographed in each of two different solvent systems. The chromatograms were run on Whatman No. 1, grade 1 untreated paper in one system with isopropyl alcohol:ethanol:HCl (3:3:1); in the other system with isopropyl alcohol:ethanol:water (2:2:1) on paper pretreated with 0.05 M phosphate buffer at pH 7.5 in 0.5 M KCl.

THIOUREA-S³⁵



C. W. Bills and A. R. Ronzio, J. Am. Chem. Soc., 72, 5510 (1950).

A. Procedure

(a) *Hydrogen Sulfide-S³⁵*. The barium sulfide-S³⁵ in 0.05 N barium hydroxide is transferred into a 40-ml. centrifuge cone, the tip of which has been drawn to a diameter of about 7 mm. and a length of about 30 mm. To this mixture is added an excess of a solution which is 0.05 N in hydrochloric acid and 0.1 N in cadmium chloride (Note 1). The precipitate of cadmium sulfide-S³⁵ is separated by decanting the clear solution after centrifugation. The precipitate, washed by repeated suspension in water and centrifugation, is dried in the cone (Note 2). The quantitative conversion of cadmium sulfide-S³⁵ to hydrogen sulfide-S³⁵ is accomplished using high-vacuum techniques. The tip of the cone, containing the active sulfide, is broken off and placed in a 200-ml. flask containing 20 g. of metaphosphoric acid. With the flask attached to a vacuum manifold and evacuated to a pressure of 5 microns, the hydrogen sulfide-S³⁵ is generated smoothly by heating the acid to boiling. The hydrogen sulfide-S³⁵ passes through a drying train containing calcium chloride and phosphorus pentoxide, and is collected in a second evacuated flask (Note 3) cooled with liquid nitrogen. From 71.9 mg. of cadmium sulfide-S³⁵ is obtained 17.8 mg. of hydrogen sulfide-S³⁵.

(b) *Thiourea-S³⁵*. The following procedure is an adaptation of the method described by Heuser.¹ The hydrogen sulfide-S³⁵ is distilled, under vacuum, into a 10-ml. flask containing a magnetic stirrer, 0.8 ml. of distilled water, 25 mg. of cyanamide (Note 4) and one drop of concentrated ammonium hydroxide. The reaction flask, which is equipped with a pressure stopcock, is closed; the mixture is maintained at 40° and stirred continuously for 24 hours (Note 5). At the end of this period, the contents of the flask are transferred with alcohol into a vacuum

sublimator and concentrated to dryness. The product is then sublimed at a pressure of 0.02–0.03 mm. and a bath temperature of 70–90 ° (Note 6). The yield (Note 7) of thiourea-S³⁵, m.p. 171–173 °, is 35 mg. (92.2%).

B. Notes

1. The quantity of reagent should be sufficient to neutralize all the base and give an acidic reaction to the mixture.
2. This intermediate preparation of cadmium sulfide-S³⁵ affords a means of preparing pure hydrogen sulfide-S³⁵.
3. This flask was equipped with a micro stopcock and was attached to the vacuum manifold with a semi-ball joint to facilitate weighing the product.
4. The cyanamide was freshly prepared to eliminate dicyandiamide.
5. The initial faint yellow color of the mixture disappeared after 6 hours, and a slight cloudiness appeared.
6. Impurities also sublimed at higher temperatures.
7. The isolation of thiourea by sublimation was quantitative. It was found advantageous to remove any grease from the dry residue with petroleum ether, in which the product was insoluble.

C. Other Preparations

Thiourea-S³⁵ has been prepared from cyanamide and hydrogen sulfide-S³⁵ in alcoholic solution at room temperature² and at the reflux temperature.³ The yields were, respectively, 79% and 67%. The same reaction at 50 ° has been reported.⁴ Thiourea-S³⁵ has also been prepared directly from barium sulfide-S³⁵, ammonium bicarbonate, and cyanamide in 92% yield.^{5,6}

Hydrogen sulfide-S³⁵ has been prepared from barium sulfide-S³⁵ by the action of: 30% phosphoric acid;⁶ 50% phosphoric acid in the presence of granulated zinc (81% yield based on barium sulfate);⁷ 6 N phosphoric acid;⁸ 6 M phosphoric acid in the presence of zinc;⁹ and dilute hydrochloric acid in the presence of aluminum turnings.² The hydrogen sulfide-S³⁵ was either absorbed in a basic medium or dried in a phosphorus pentoxide tube and collected in a trap cooled with liquid nitrogen.

¹R. V. Heuser, U. S. 1,991,852 (1935); Chem. Abstracts, 29, 2180 (1935).

²J. Bell and K. A. MacDonald, J. Chem. Soc., 1951, 1930.

³C. Walling, J. Am. Chem. Soc., 70, 2561 (1948).

⁴J. J. Bezem, F. Brunnekreeft, M. J. E. Ernsting, J. Lever and W. Th. Nauta, Acta Endocrinol., 3, 151 (1949).

⁵V. I. Maimind, M. N. Shchukina and T. F. Zhukova, Zhur. Obshchei Khim. (J. Gen. Chem.), 22, 1279 (1952).

⁶Y. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, Doklady Akad. Nauk S. S. S. R., 91, 1129 (1953).

⁷J. C. Bournsnel, G. E. Francis and A. Wormall, Biochem. J., 40, 743 (1946).

Using sulfuric- S^{35} acid and an excess of aniline, Pressman⁵ obtained an 18% yield of sulfanilic- S^{35} acid, based on isotope. By the isotopic dilution method it was shown that 0.7% of the initial activity was present as *meta*-isomer and 2% as *ortho*-isomer, together with the 18% of *para*-isomer. Ingraham⁶ employed an exchange reaction between aniline acid sulfate and sulfuric- S^{35} acid, followed by the baking procedure.⁷ The yields of sulfanilic- S^{35} acid reported were 95% for 60 mg., and 60 to 70% for 2 to 5-mg. amounts. The product contained less than 0.2% of *ortho*- or *meta*-isomer, and two recrystallizations from 2% sodium sulfate solution reduced the free $S^{35}O_4^{=}$ to less than 0.2% of the total S^{35} in the product. According to Ingraham,⁸ not more than 0.6% of sulfanilic acid sulfur exchanged with sulfur from free sulfate in acid, basic or neutral solution in 55 days at 80° .

N^4 -Acetyl- N^1 -2-pyridylsulfanilamide- S^{35} and sulfapyridine- S^{35} have been prepared⁹ in 57% and 55% yields, respectively.

¹H. Erdmann, cited by L. Vanino, *Handbuch der Präparativen Chemie*, 2, 655, 2nd. ed., Stuttgart: Enke.

²G. Schroeter, Ber., 39, 1559 (1906).

³R. Winterbottom, J. Am. Chem. Soc., 62, 160 (1940).

⁴M. Sonenberg, A. S. Keston, W. I. Money and R. W. Rawson, J. Clin. Endocrinol. and Metabolism, 12, 1269 (1952).

⁵D. Pressman, H. N. Eisen, M. Siegel, P. J. Fitzgerald, B. Sherman and A. Silverstein, J. Immunol., 65, 559 (1950).

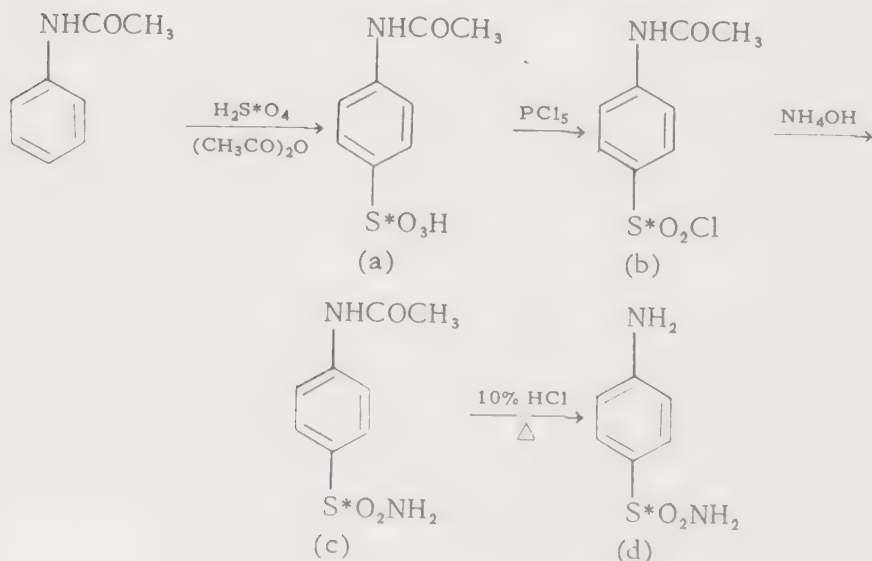
⁶J. S. Ingraham, J. Am. Chem. Soc., 74, 2433 (1952).

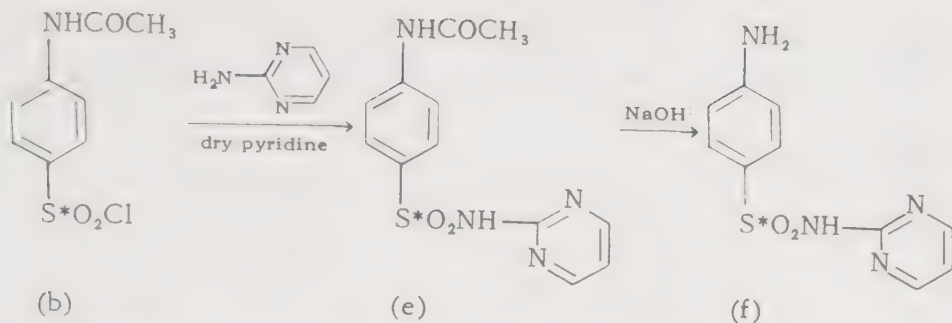
⁷W. Huber, Helv. Chim. Acta, 15, 1372 (1932).

⁸J. S. Ingraham, J. Inf. Diseases, 89, 109 (1950).

⁹Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, Doklady Akad. Nauk S.S.S.R., 91, 1129 (1953).

SULFADIAZINE- S^{35} (N^1 -2-Pyrimidinylsulfanilamide- S^{35})





P. J. Byrne, Jr., A. A. Alberts and J. E. Christian, *J. Am. Pharm. Assoc., Sci. Ed.*, 42, 77 (1953).

A. Procedure (Note 1)

(a) *N*-Acetylsulfanilic-S³⁵ Acid, (4-Acetamidobenzenesulfonic-S³⁵ Acid). The sulfuric-S³⁵ acid (about 2 mc.) in a dilute solution of hydrochloric acid is evaporated to dryness in a 30-ml. beaker, using an infrared lamp. Two ml. of concentrated sulfuric acid is added as carrier, and the acid is cooled while 4 ml. of acetic anhydride is added slowly. Then, 2 g. of acetanilide is added in small portions with stirring, and the resultant mixture is heated to 93-100° on a steam-bath for 30 minutes; *N*-acetylsulfanilic-S³⁵ acid separates. The reaction mixture is cooled in an ice-bath and diluted with 10 ml. of cold acetone, and the product is collected on a Büchner funnel. After the product is washed with a small amount of cold acetone, it is transferred to a porous plate and dried under an infrared lamp. The yield of *N*-acetylsulfanilic-S³⁵ acid is 2.99 g. (94%) (Note 2).

(b) *N*-Acetylsulfanilyl- S^{35} Chloride, (4-Acetamidobenzenesulfonyl- S^{35} Chloride). With ice-cooling, 5.98 g. of phosphorus pentachloride is added to 2.99 g. of *N*-acetylsulfanilic- S^{35} acid, and the reactants are ground together. After the initial reaction subsides, the mixture is gently heated until a homogeneous liquid mass is obtained. The solution is cooled in an ice-bath, and approximately 10 g. of crushed ice is added to decompose the excess phosphorus pentachloride. *N*-Acetylsulfanilyl- S^{35} chloride is precipitated (Note 3).

(c) *N*⁴-Acetylsulfanilamide-*S*³⁵, (4-Acetamidobenzenesulfonamide-*S*³⁵). The above suspension of *N*-acetylsulfanilyl-*S*³⁵ chloride is cooled in an ice-bath. 10 ml. of concentrated ammonium hydroxide is slowly added, and the mixture is warmed on a steam-bath for 30 minutes. The solution is cooled, and the pH is adjusted to 3 with dilute sulfuric acid. The product is collected, washed with a small amount of cold water, transferred to a porous plate and dried with an infrared lamp. The yield of crude product, m.p. 214–218°, is 1.64 g. (54.8%). After recrystallization from water, 1.29 g. (43.1%) of 4-acetamidobenzenesulfonamide-*S*³⁵, m.p. 219°, is obtained (Note 4).

(d) *Sulfanilamide-S³⁵*, (4-Aminobenzenesulfonamide-S³⁵). Crude *N*⁴-acetylsulfanilamide-S³⁵, 2.13 g., is heated on a steam-bath with 5 ml. of 10%

hydrochloric acid for 1 hour. The mixture is cooled and then neutralized in 2 steps. First, the pH is adjusted to approximately 5 with 5 *N* sodium hydroxide; then, neutralization is completed with 10% sodium bicarbonate solution. After the solution is chilled for a time, the product is collected, washed with a small quantity of cold water, transferred to a porous plate and dried under an infrared lamp. After recrystallization from water, the yield of sulfanilamide-S³⁵, m.p. 164°, is 1.04 g. (36.6%).

(e) *N*⁴-Acetyl-*N*¹-2-pyrimidinylsulfanilamide-S³⁵. To a cold suspension of 1.05 g. of 2-aminopyrimidine in 5 ml. of dry pyridine is added gradually 2.98 g. of dry, crude *N*-acetylsulfanilyl-S³⁵ chloride, with stirring (Note 5). The reaction mixture is heated on a steam-bath for one hour. Then the pyridine is removed under diminished pressure in the temperature range of 27–42°; water is added periodically to maintain a minimum volume of about 25 ml. The yield of crude *N*⁴-acetyl-*N*¹-2-pyrimidinylsulfanilamide-S³⁵, which separates as a yellow solid, m.p. 217°, is 1.5 g. (46.5%). After recrystallization from 60% acetic acid, the product melts at 257–258°.

(f) *Sulfadiazine-S*³⁵, (*N*¹-2-Pyrimidinylsulfanilamide-S³⁵). *N*⁴-Acetyl-*N*¹-2-pyrimidinylsulfanilamide-S³⁵, 1.5 g., is dissolved in 4.5 ml. of 10% sodium hydroxide, and the solution is refluxed for 1 1/2 hours. The solution is cooled, diluted with 20 ml. of cold water, filtered, and neutralized with dilute hydrochloric acid. The white precipitate is collected, washed with cold water and dried under an infrared lamp. The yield (Note 6) of crude sulfadiazine-S³⁵, m.p. 218°, is 1.12 g. (34.7%). After recrystallization first from 60% acetic acid and then from 50% ethanol, the product melts at 255°.

B. Notes

1. With the exception of the two pyrimidine derivatives (e) and (f), the procedures used in the following preparations are similar to those described by Fingl¹ and by Klotz and Melchior.³

2. The yields from two preparations were 2.99 g. and 2.84 g., 94.1% and 89.3%, respectively, based on acetanilide.

3. The acetylsulfanilyl-S³⁵ chloride was not isolated.

4. These yields are based on *N*-acetylsulfanilic-S³⁵ acid.

5. The addition is made at such a rate that the temperature does not exceed 55°.

6. The yield is based upon 2-aminopyrimidine.

C. Other Preparations

Sulfanilamide-S³⁵ has been prepared in 25–35% yield¹ from sulfuric-S³⁵ acid and acetanilide. Sodium *N*-acetylsulfanilate-S³⁵ (sodium 4-aceta-

midobenzenesulfonate-S³⁵) was prepared as an intermediate and isolated in 97% yield.

Using a procedure similar to that described, Markova² prepared: *N*-acetylsulfanilic-S³⁵ acid (75% yield based on sulfuric-S³⁵ acid), sodium *N*-acetylsulfanilate-S³⁵ (100% yield), *N*-acetylsulfanilyl-S³⁵ chloride (76% yield), *N*⁴-acetylsulfanilamide-S³⁵ (74% yield) and sulfanilamide-S³⁵ (43% yield).

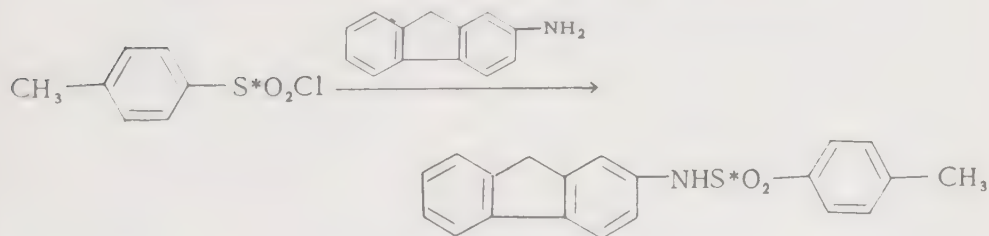
Klotz and Melchior³ have also prepared sulfanilamide-S³⁵, m.p. 161-162° (cor.), *via* the described intermediates. The over-all yield based on sodium sulfide-S³⁵ was 31%.

¹E. G. Fingl, J. E. Christian and L. D. Edwards, J. Am. Pharm. Assoc., Sci. Ed. 39, 693 (1950).

²Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, Doklady Akad. Nauk S.S.S.R., 91, 1129 (1953).

³I. M. Klotz and J. B. Melchior, Arch. Biochem., 21, 35 (1949).

N-2-FLUORENYL-*p*-TOLUENESULFONAMIDE-S³⁵



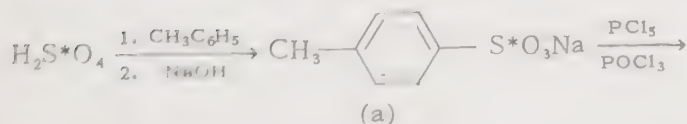
F. E. Ray and M. F. Argus, Cancer Research, 11, 783 (1951).

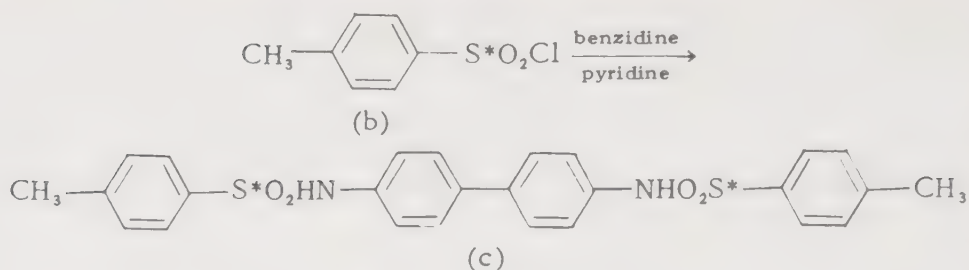
Procedure

According to the procedure of Campbell,¹ a mixture of 5 g. of 2-fluorenamine, 6 g. of *p*-toluenesulfonyl-S³⁵ chloride and pyridine is heated under reflux for 1 hour. The resulting solution is poured into cold, dilute hydrochloric acid. The solid product is collected and recrystallized from either glacial acetic acid or carbitol. The yield of colorless prisms, m.p. 157-158°, is 7 g.

¹N. Campbell, W. Anderson and J. Gilmore, J. Chem. Soc., 1940, 446.

4',4''-BI(*p*-TOLUENESULFONANILIDE)-S₂³⁵ [*N,N'*-Di(*p*-toluenesulfonyl)benzidine-S₂³⁵]





F. E. Ray and L. Soffer, *J. Org. Chem.*, **15**, 1037 (1950).

A. Procedure

(a) *Sodium p-Toluenesulfonate-S³⁵*. A three-necked flask is equipped with a thermometer, a mercury-sealed stirrer and a side-arm water trap attached to a vertical condenser, which is protected against atmospheric moisture. The water trap is packed with a mixture of calcium chloride and glass beads to 0.5 inch below the side-arm, and then filled with pure toluene (Note 1) until the liquid level is slightly below the level of calcium chloride. In the flask, a mixture of 10 ml. (0.18 mole) of sulfuric-S³⁵ acid and 20 ml. (0.19 mole) of toluene is heated, with stirring, at such a rate that the temperature rises to 195° in one hour. The flame is removed, the solution is cooled to 100°, 3-4 ml. of toluene is added with thorough mixing, and heating is resumed. This process is repeated 4 or 5 times, with simultaneous removal of water from the trap until no more water is apparent on heating the mixture. The side-arm is partially emptied, and the flask is heated to 150° to remove excess toluene (Note 2).

The hot reaction mixture is washed into 100 ml. of water and neutralized by the careful addition of sodium hydroxide. After the addition of 30 g. of sodium chloride, the mixture is heated to boiling, and water is added, if necessary, to dissolve the salt. The solution is filtered, and the filtrate is cooled in an ice-bath. The sodium *p*-toluenesulfonate-S³⁵ is collected, washed with 25 ml. of saturated sodium chloride solution and pressed dry. The product is dissolved in 50 ml. of hot water, 10 g. of sodium chloride is added, and sufficient water, 20-25 ml., is added to bring the latter into solution. The solution is treated with 0.5 g. of charcoal, filtered while hot and concentrated to 70 ml. After crystallizing in the cold, the yield of colorless product is 19.7-22 g. (56.6-63.2%) (Note 4).

(b) *p-Toluenesulfonyl-S³⁵ Chloride*. A mixture of 10 g. (0.051 mole) of sodium *p*-toluenesulfonate-S³⁵ (dried at 140°), 5 g. (0.024 mole) of phosphorus pentachloride, and 10 ml. (0.109 mole) of phosphoryl chloride is refluxed (160°) for 1 1/2 hours, with frequent agitation. Excess phosphoryl chloride is removed under partial vacuum, and the residue is washed into 400 ml. of ice-water. The nearly white solid is washed

with a little cold water and dried over phosphorus pentoxide in a desiccator. The product, m.p. 69° , (Note 5) weighs 8.6 g. (89.6%).

(c) 4',4'''-Bi(*p*-toluenesulfonanilide)- S_2^{35} , [N,N'-Di(*p*-toluenesulfonyl)-benzidine- S_2^{35}]. To a mixture of 6.1 g. of purified benzidine¹ and 14.5 g. of *p*-toluenesulfonyl- S^{35} chloride is added 20 ml. of dry pyridine. The mixture is refluxed for 10-15 minutes, poured into ice-water and stirred until the product crystallizes.² The crude product, when dry, weighs 9.4 g. and melts at $236-239^{\circ}$. After several recrystallizations from aqueous acetone, with charcoal treatment, the yield of product, m.p. 248° (Note 6), is 7.6 g. (40.6%).

B. Notes

1. Ordinary toluene may contain thiophene derivatives which cause darkening of the reaction mixture. These are removed by treatment with sulfuric acid.³

2. The total time required was 5 hours.

3. Filtration removes 2-3 g. of yellow, waxy 4,4'-sulfonylditoluene- S_2^{35} .

4. The sulfonate was identified by conversion to *p*-toluidine *p*-toluenesulfonate- S^{35} , m.p. 197° .⁴

5. This value is in agreement with the literature.⁵

6. Willstätter⁶ reported 243° .

C. Other Preparations

Meader and Fries⁷ have reported the preparation of a sodium alkylbenzenesulfonate- S^{35} by treatment of the alkylbenzene (1.03 mmoles) with a solution of barium sulfate- S^{35} in 20% fuming sulfuric acid at 0° , in the presence of phosphorus pentoxide. After neutralization of the acid with sodium hydroxide and purification of the sodium salt, the yield was 93% (isotopic yield, 62%).

¹C. Weygand, *Organic Preparations*, Interscience, New York, 1945, p. 238.

²A. I. Vogel, *Practical Organic Chemistry*, Longmans, Green and Co., New York, 1948, p. 625.

³L. F. Fieser, *Experiments in Organic Chemistry*. 2nd. ed., Heath and Co., New York, p. 364.

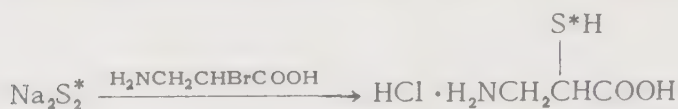
⁴*Idem*, p. 140.

⁵T. S. Patterson and J. Frew, *J. Chem. Soc.*, 89, 332 (1906).

⁶R. Willstätter and L. Kalb, *Ber.*, 37, 3772 (1904).

⁷A. L. Meader, Jr. and B. A. Fries, *Ind. Eng. Chem.*, 44, 1636 (1952).

ISOCYSTEINE-S³⁵ HYDROCHLORIDE
(3-Amino-2-mercaptopropionic-S³⁵ Acid Hydrochloride)



D. D. Dziwiatkowski and W. J. Wingo, *Proc. Soc. Exptl. Biol. Med.*, 70, 448 (1949).

A. Procedure

Isocystiene-S³⁵ hydrochloride is prepared from 2-bromo-β-alanine and sodium disulfide-S³⁵ by adaptation of the following procedure of Schöberl and Braun.¹

To a solution of 5 g. of 2-bromo-β-alanine hydrobromide in 8 ml. of water, which is neutralized with 22 ml. of 1N sodium hydroxide solution (to pH 6.8), is added 12 ml. of sodium disulfide solution (Note 1). The reddish-brown solution, which is sometimes turbid, is flushed with nitrogen and kept at room temperature under a nitrogen atmosphere for 12–15 hours (Note 2). The solution is shaken with activated carbon and filtered, and the filtrate is concentrated to half its volume *in vacuo*. Then the solution is acidified with 30 ml. of concentrated hydrochloric acid and reduced with 4 g. of powdered tin. The resultant solution is diluted to 400 ml., saturated with hydrogen sulfide and filtered. The colorless filtrate is evaporated to dryness at 45°, *in vacuo*. The yellowish-colored residue is dissolved in 30 ml. of water and treated with 70–80 ml. of saturated mercuric chloride solution. The white, granular mercury mercaptide precipitates, and the mixture is cooled overnight. The product is collected, washed with water, dissolved in 40–50 ml. of 10% hydrochloric acid, and treated with hydrogen sulfide to free it of mercury. The solution is filtered, and the clear filtrate is concentrated *in vacuo* to a clear oil, which crystallizes upon standing in a vacuum desiccator. After the crystalline isocysteine hydrochloride is washed with acetone and ether, it melts at 137–139° and weighs 1.73 g. (54.8%) (Note 3).

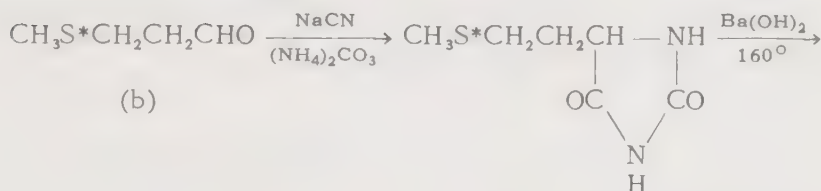
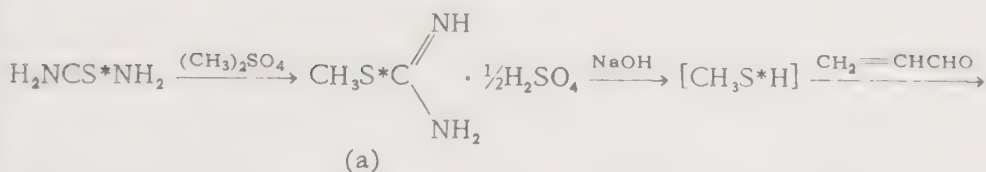
B. Notes

1. The disulfide solution contained 325 mg. of polysulfide sulfur.
2. At this time the color of the mixture is yellow, and the precipitated sulfur has clustered together.
3. In the isotopic synthesis, using the same amounts of starting materials, the yield was 0.88 g. (25%).

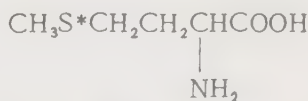
¹A. Schöberl and H. Braun, *Ann.*, 542, 274 (1939).

METHIONINE-S³⁵
[2-Amino-4-(methylthio)butyric-S³⁵ Acid]

METHOD I



(c)



V. I. Maimind, M. N. Shchukina and T. F. Zhukova, Zhur. Obshchei Khim. (J. Gen. Chem.), 22, 1279 (1952).

A. Procedure

(a) *S*-Methylisothiuronium-S³⁵ Sulfate. The procedure employed is an adaptation of the method described in *Organic Syntheses*¹ (Note 1). The average yield of *S*-methylisothiuronium-S³⁵ sulfate is 78%.

(b) 3-(Methylthio)propionaldehyde-S³⁵ (Note 2). A mixture of 0.5 g. of *S*-methylisothiuronium-S³⁵ sulfate and 0.72 ml. of 5 *N* sodium hydroxide, in a small distilling flask, is heated gently over a small flame. The resultant steady current of methanethiol-S³⁵ is passed through dilute sulfuric acid (1:2) and calcined, fine-grained calcium chloride and is then absorbed in 1.5-2.0 ml. of acrolein. The temperature of the acrolein, which contains traces of triethylamine (Note 3), is kept below -2°. The reaction requires about 40 minutes. More heat is applied to the generator toward the end of the reaction, and residual methanethiol-S³⁵ is swept into the acrolein with a stream of nitrogen. The reaction mixture is transferred to a small distillation flask with ether. The excess acrolein and ether are removed under reduced pressure, and the residue is distilled. The yield is 0.26 g. (70%); b.p. 80° (30 mm.) and 66° (17 mm.).

(c) 5-[2-(Methylthio)ethyl]hydantoin-S³⁵. This compound is prepared according to an adaptation of the method of Pierson, *et al.*,² which

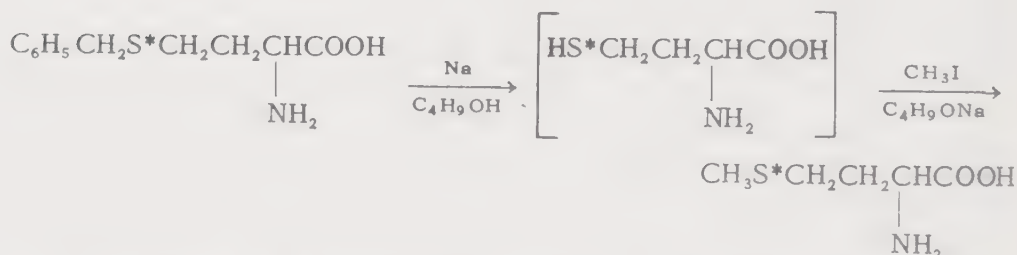
employs the Bücherer hydantoin synthesis, as in the following example.

A mixture of 26 g. (0.25 mole) of 3-(methylthio)propionaldehyde, 113 g. (1.17 moles) of finely divided ammonium carbonate, 24.5 g. (0.5 mole) of sodium cyanide, 335 ml. of ethanol and 335 ml. of water is maintained at 50–55° for 4 hours with constant stirring. The light yellow reaction mixture is filtered; the filtrate is concentrated at 60° to a volume of 300 ml., acidified with 50 ml. of concentrated hydrochloric acid and heated for five minutes at 90° (Note 4). After cooling the solution, the product is collected and dried. The yield of 5-[2-(methylthio)ethyl]hydantoin, m.p. 103–105°, is 34 g. (79%) (Note 5).

On the millimole scale the yield of 5-[2-(methylthio)ethyl]hydantoin-S³⁵, m.p. 103–104°, does not exceed 65–70%.

(d) *Methionine-S³⁵*, [2-Amino-4-(methylthio)butyric-S³⁵ Acid]. According to the procedure of Livak, *et al.*,³ 1.3 g. of finely powdered barium hydroxide octahydrate and 0.42 g. of 5-[2-(methylthio)ethyl]hydantoin-S³⁵, dissolved in 7.7 ml. of hot water, are placed in a tube. The tube is sealed and heated in an oscillating bath at 155–165°. The mass solidifies within 30 minutes, and heating is discontinued. After the barium carbonate is collected on a filter, the filtrate is shaken with 0.22 g. of ammonium carbonate, again filtered and then evaporated to dryness in *vacuo* at 60°. The dry residue is suspended in 2–3 ml. of absolute ethanol and collected on a filter with vacuum. The yield of crude methionine-S³⁵ is 0.28 g. (93%). After recrystallization from aqueous alcohol, the yield of pure product (Note 6) is 0.22–0.24 g. (Note 7).

METHOD II



A. M. Seligman, A. M. Rutenburg and H. Banks, *J. Clin. Invest.*, 22, 275 (1943).

A. Procedure

2-Amino-4-(benzylthio)butyric-S³⁵ acid, 1.56 g., is suspended in 30 ml. of butanol. With the alcohol refluxing, 1.6 g. of sodium is added in small pieces during 2.5 hours. The solution of 2-amino-4-mercaptobutyric-S³⁵ acid is cooled to –10°, 6.9 g. (0.0485 mole) of methyl iodide is added, and the temperature is kept at 10° for 30 minutes. Then water is

added, and the solution is neutralized and concentrated, *in vacuo*, to a small volume. The yield of methionine-S³⁵, which crystallizes in white leaflets, is 21%.

B. Notes

1. The methyl sulfate used was first neutralized and distilled.
2. It is convenient to carry out this reaction in an apparatus for the micro determination of methoxyl groups.⁴
3. The triethylamine is added in a platinum wire loop; if a larger amount (1 drop) is used, the acrolein may polymerize.
4. The mixture is heated to cyclize hydantoic acid which is present in small amounts.
5. The melting point remained unchanged after recrystallization of the product from ethanol.
6. The melting point of pure methionine is 268–270° with decomposition.³
7. The over-all yield based on barium sulfide-S³⁵ is 31.8%.

C. Other Preparations

Methionine-S³⁵ has been prepared by reducing 2-amino-4-(benzylthio)butyric-S³⁵ acid with sodium in liquid ammonia⁵ and treatment of the resulting intermediate with methyl iodide⁶ or methyl iodide in dry ether.⁷

Methionine-S³⁴ has been prepared⁸ from 2-amino-4-(benzylthio)butyric-S³⁴ acid by a similar procedure.

S-Methylisothiuronium-S³⁵ sulfate (76% yield), methanethiol-S³⁵ (100% yield), 3-(methylthio)propionaldehyde-S³⁵ (72% yield), 5-[2-(methylthio)ethyl]hydantoin-S³⁵ (60% yield), and methionine-S³⁵ (71% yield) have been prepared⁹ essentially according to the procedures described in Method I.

¹Organic Syntheses, Coll. Vol. II, Wiley, New York, 1943, p. 411.

²E. Pierson, M. Giella and M. Tishler, J. Am. Chem. Soc., 70, 1450 (1948).

³J. E. Livak, E. C. Britton, J. C. Vander Weele and M. F. Murray, J. Am. Chem. Soc., 67, 2218 (1945).

⁴S. Edlbacher, Z. physiol. Chem., 101, 278 (1918).

⁵V. du Vigneaud, L. F. Audrieth and H. S. Loring, J. Am. Chem. Soc., 52, 4500 (1930).

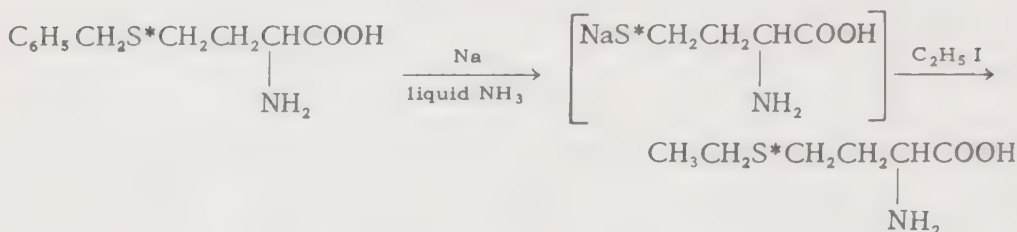
⁶H. Tarver and C. L. A. Schmidt, J. Biol. Chem., 130, 67 (1939).

⁷Idem, 146, 69 (1942).

⁸G. W. Kilmer and V. du Vigneaud, *ibid.*, 154, 247 (1944).

⁹Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, Doklady Akad. Nauk S.S.S.R., 91, 1129 (1953).

2-AMINO-4-(ETHYLTHIO)BUTYRIC-S³⁵ ACID
(Ethionine-S³⁵)



J. A. Stekol and K. Weiss, *J. Biol. Chem.*, **185**, 577 (1950).

A. Procedure

The preparation of ethionine-S³⁵ by the reduction of 2-amino-4-(benzylthio)butyric-S³⁵ acid with sodium in liquid ammonia and treatment of the resulting sodium salt with ethyl iodide is similar to the preparation of methionine-S³⁵ from the same intermediates.^{1,2} The following procedure is taken from the preparation of ethionine described by Dyer.³

To 200 ml. of liquid ammonia in a 3-necked flask equipped with a mercury-sealed stirrer is added 18 g. of 2-amino-4-(benzylthio)butyric acid. The flask is cooled in a Dry Ice-trichloroethylene bath. Sodium is introduced in small portions until a blue color persists for several minutes (Note 1). To this solution 10 ml. of ethyl bromide is added dropwise. Then the cold-bath is removed, and the liquid ammonia is allowed to evaporate. The residue is treated with 50 ml. of water, the solution is filtered, and the filtrate is made just acidic to litmus with hydriodic acid. After the solution is cooled in an ice-bath, the crude product, 9.8 g. (75%), is collected and dried. Recrystallization of the crude product from 100 ml. of water affords 8 g. of ethionine (Note 2), which begins to melt at 272° (Note 3) and effervesces at 284° (cor.).

B. Notes

1. The blue color indicates excess sodium; about 4 g. of sodium is required.

2. The pure product should be free of halogen and disulfide.

3. Heating was at the rate of 20° per minute.

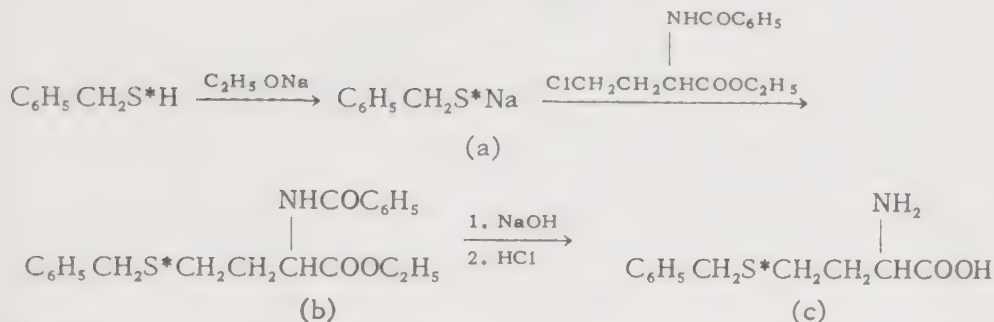
¹H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **130**, 67 (1939); *ibid.*, **146**, 69 (1942).

²G. W. Kilmer and V. du Vigneaud, *ibid.*, **154**, 247 (1944).

³H. M. Dyer, *ibid.*, **124**, 519 (1938).

2-AMINO-4-(BENZYLTHIO)BUTYRIC-S³⁵ ACID
(S-Benzylhomocysteine-S³⁵)

METHOD I



H. Tarver and C. L. A. Schmidt, J. Biol. Chem., 146, 69 (1942).

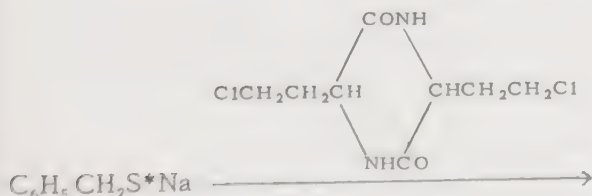
A. Procedure

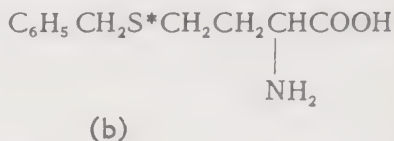
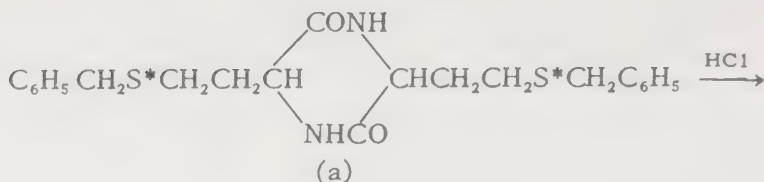
(a) *Sodium α -Toluenethiolate-S³⁵*. α -Toluenethiol-S³⁵, 0.53 g. (4.3 mmoles), is dissolved in 1 ml. of absolute methyl alcohol containing 0.15 g. (6.5 mmoles) of sodium.

(b) *Ethyl 2-Benzamido-4-(benzylthio)butyrate-S³⁵*. With the sodium α -toluenethiolate-S³⁵ solution at its boiling point, 1.4 g. (5.2 mmoles) of ethyl 2-benzamido-4-chlorobutyrate¹⁻⁴ is added. The mixture is refluxed for 10 minutes to complete the reaction.

(c) *2-Amino-4-(benzylthio)butyric-S³⁵ Acid, (S-Benzylhomocysteine-S³⁵)*. To the above mixture 60 ml. of 0.25 N sodium hydroxide is added, and the hydrolysis of the ester group is effected by refluxing the mixture for 15 minutes. The 2-benzamido-4-(benzylthio)butyric-S³⁵ acid is not isolated, and, after the addition of 80 ml. of constant boiling hydrochloric acid, the mixture is refluxed for 5 hours. Benzoic acid is filtered from the cold solution and washed with ice-water. After the excess hydrochloric acid is distilled off, the solution is adjusted to pH 5.5 to 6. The precipitate of S-benzylhomocysteine-S³⁵ is collected and washed with ice-water, 95% ethanol and ether. The yield of dry product, m.p. 235–239° (cor.) with decomposition, is 0.718 g. (75% based on α -toluenethiol-S³⁵).

METHOD II





J. L. Wood and H. R. Gutmann, *J. Biol. Chem.*, **179**, 535 (1949).

A. Procedure

(a) *3,6-Bis[2-(benzylthio)ethyl]-2,5-piperazinedione-S₂³⁵*. According to the procedure of Snyder and Chiddix,⁵ a suspension of 240 mg. of 3,6-bis(2-chloroethyl)-2,5-piperazinedione² in 5 ml. of absolute ethanol is added to a solution of 1.1 mmole of sodium benzylthiolate-S³⁵ in 5 ml. of ethanol. After the mixture is refluxed for 1 hour, the solvent is distilled off, leaving the product as a nearly dry residue (Note 1).

(b) *S-Benzyl-DL-homocysteine-S³⁵*. To the residue of 3,6-bis[2-(benzylthio)-ethyl]-2,5-piperazinedione-S₂³⁵ is added 10 ml. of 9 N hydrochloric acid. The suspension is heated under reflux at 110–120° for 7 hours. The solution is then cooled to room temperature, filtered with charcoal and concentrated nearly to dryness *in vacuo*. To the residue is added 5 ml. of distilled water, and the solution is again concentrated *in vacuo*. The residue is dissolved in 10 ml. of distilled water and neutralized with concentrated ammonium hydroxide. The precipitate is collected and washed successively with distilled water, cold absolute ethanol and cold ether. The yield of *S*-benzyl-DL-homocysteine-S³⁵ is 55.3 mg. (25%) (Note 2).

(c) *S-Benzyl-D-homocysteine-S³⁵* (Note 3). To 9.87 mg. of *S*-benzyl-DL-homocysteine-S³⁵ is added 100 mg. of unlabeled *S*-benzyl-D-homocysteine. The mixture is dissolved in 5 ml. of *N* hydrochloric acid and reprecipitated by the addition of 5 ml. of *N* sodium hydroxide. After the pH is adjusted to 7, the mixture is placed in an ice-bath for several hours. The precipitate is collected and washed with cold absolute ethanol and cold ether. After it is dried over phosphorus pentoxide *in vacuo*, the product weighs 91 mg. After three such recrystallizations from 10-ml. portions of *N* hydrochloric acid, the removal of L-isomer is estimated to be complete (Note 4), and the weight of *S*-benzyl-D-homocysteine-S³⁵ is 25 mg.

B. Notes

1. The melting point of this compound, after recrystallization from ethanol, given in the literature⁵ is 173–174° (cor.).

2. Snyder and Chiddix⁵ list the melting point of this compound, prepared in the same manner, as 226–230° (cor.). The *N*-acetyl derivative, prepared in a preliminary experiment by Wood and Gutmann, according to the procedure of du Vigneaud and Irish,⁶ melted at 115°.

3. The use of optical activity or radioactivity measurements can not be used to demonstrate the removal of one optical isomer from the other by fractional crystallization,⁷ since optical activity measurements are too gross to detect a minute amount of L-isomer in the D-fraction, and both isomers would have the same specific activity. The separation of labeled optical isomers by isotopic dilution offers a solution to the problem. Fractional crystallization to isolate a single, labeled enantiomorph can then be followed by radioactivity measurements until a constant specific activity is obtained.

4. It was possible to calculate the percentage removal of L-isomer from the specific activity of the product after each recrystallization. *N*-acetyl-S-benzyl-D-homocysteine-S³⁵ was prepared⁶ with no change in the specific activity of the sulfur.

C. Other Preparations

S-Benzylhomocysteine-S³⁵ has been prepared⁸ from methionine-S³⁵ according to a procedure described by Stekol.⁹ It has also been prepared by the hydrolysis of ethyl α -[2-(benzylthio)ethyl]-1,3-dioxo-2-isoindolinemalonate-S³⁵.^{10,11}

2-Amino-4-(benzylthio)butyric-3,4-C₂¹³-S³⁴ acid (C₂¹³-S-benzylhomocysteine-S³⁴) has been prepared by hydrolysis of the corresponding 1,3-dioxo-2-isoindolinemalonate.¹²

⁵E. M. Hill and W. Robson, *Biochem. J.*, **30**, 248 (1936).

²H. R. Snyder, J. H. Andreen, G. W. Cannon and C. F. Peters, *J. Am. Chem. Soc.*, **64**, 2082 (1942).

³H. R. Snyder and G. W. Cannon, *ibid.*, **66**, 511 (1944).

⁴J. E. Livak, E. C. Britton, J. C. Vander Weele and M. F. Murray, *ibid.*, **67**, 2218 (1945).

⁵H. R. Snyder and M. E. Chiddix, *ibid.*, **66**, 1000 (1944); *ibid.*, **66**, 1002 (1944).

⁶V. du Vigneaud and O. J. Irish, *J. Biol. Chem.*, **122**, 349 (1937–1938).

⁷V. du Vigneaud and W. I. Patterson, *ibid.*, **109**, 97 (1935).

⁸H. R. Gutmann and J. L. Wood, *ibid.*, **189**, 473 (1951).

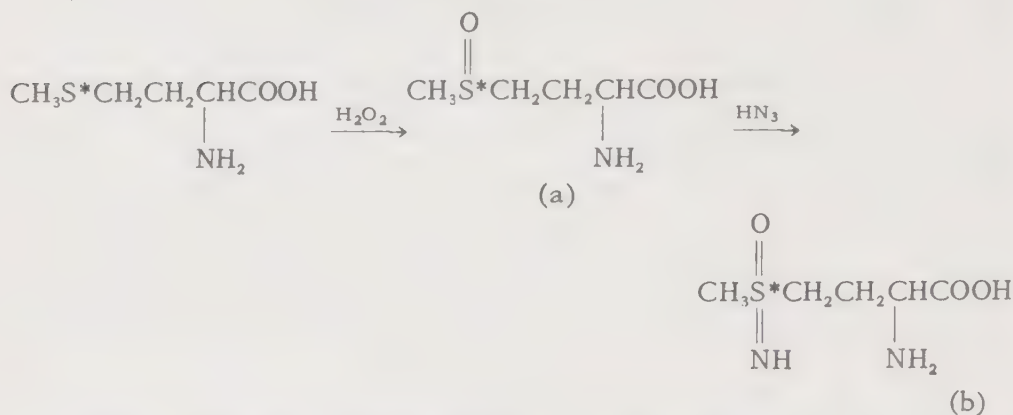
⁹J. A. Stekol, *ibid.*, **140**, 827 (1941).

¹⁰A. M. Seligman, A. M. Rutenburg and H. Banks, *J. Clin. Invest.*, **22**, 275 (1943).

¹¹H. Tarver and C. L. A. Schmidt, *J. Biol. Chem.*, **130**, 67 (1939).

¹²G. W. Kilmer and V. du Vigneaud, *ibid.*, **154**, 247 (1944).

L-3-AMINO-3-CARBOXYPROPYL METHYL SULFOXIMINE-S³⁵
(L-Methionine-S³⁵ Sulfoximine)



J. S. Roth, A. Wase and L. Reiner, *Science*, **115**, 236 (1952).

A. Procedure

(a) *L*-2-Amino-4-(methylsulfinyl)butyric-S³⁵ Acid, (*L*-Methionine-S³⁵ Sulfoxide). *L*-Methionine-S³⁵ (1.04 g.) is converted to *L*-methionine-S³⁵ sulfoxide (0.96 g.) by adapting the procedure of Toennies and Kolb,¹ which is as follows.

In a mixture of 220 mmoles of concentrated hydrochloric acid, 150 ml. of water, and 250 ml. of methanol is dissolved 200 mmoles of methionine. To this solution is added 240 mmoles of hydrogen peroxide (Note 1), with cooling, and water to make the volume about 500 ml. (Note 2). After the solution is well mixed and allowed to stand for 20 to 30 minutes, 230 mmoles of amylamine and methyl alcohol, equal to about one-half of the total volume of the reaction mixture, are added. The solution is filtered, and to the filtrate is added 3 volumes of acetone (about 2400 ml.). After about 10 minutes the supernatant liquid becomes clear, and the precipitated sulfoxide is collected (Note 3). After the product is resuspended six times in 500-ml. portions of acetone (Note 4), it is dried in air and finally heated for 1 hour at 100°. The yield is 95% of theoretical (Note 5).

(b) *L*-3-Amino-3-carboxypropyl Methyl Sulfoximine-S³⁵, (*L*-Methionine-S³⁵ Sulfoximine). The following procedure is an adaptation of the method of Misani and Reiner² with several modifications. The methionine-S³⁵ sulfoxide, 0.96 g. (0.007 moles), is placed in a 15-ml. 3-necked flask equipped with dropping funnel, stirrer and gas delivery tube (Note 6). The flask is cooled in an ice-bath, and 2 ml. of concentrated sulfuric acid is added with stirring. When most of the sulfoxide has dissolved, the temperature is raised to 45° by means of a water-bath, and 0.60 g. (0.014 mole) of hydrazoic acid, dissolved in chloroform, is added during

1 hour (Note 7). When all the hydrazoic acid has been added, stirring is continued for several hours, and the mixture is allowed to stand overnight (Note 8). The reaction mixture is poured into a small amount of cracked ice and adjusted to pH 5.5 with barium carbonate. The barium sulfate precipitate is collected on a filter and washed with hot water until the washings give a negative ninhydrin test. The combined filtrate and washings are then evaporated *in vacuo* to about 5 ml. Any precipitate is removed, and the filtrate is evaporated nearly to dryness. The residue is dissolved in a minimum of hot water, an equal volume of methyl alcohol is added, and the solution is cooled overnight. After the crystals of L-methionine-S³⁵ sulfoximine are recrystallized from a small quantity of hot water, the yield is 0.434 g.

B. Notes

1. The hydrogen peroxide, added in about 20% excess, was a 30% solution.

2. This is done in a volumetric flask if it is desired to follow the oxidation by titration.

3. If left standing for a long period, the precipitate is much more difficult to filter and may discolor to some degree.

4. A test for chloride ion becomes negative, indicating absence of amylammonium chloride.

5. A chromatogram was run on the product, using 0.05% butanol-acetic acid; this gave R_f ascending 0.13. The R_f of pure methionine sulfoxide is 0.13.

6. The outer end of the tube is placed several millimeters below the surface of some water in a beaker.

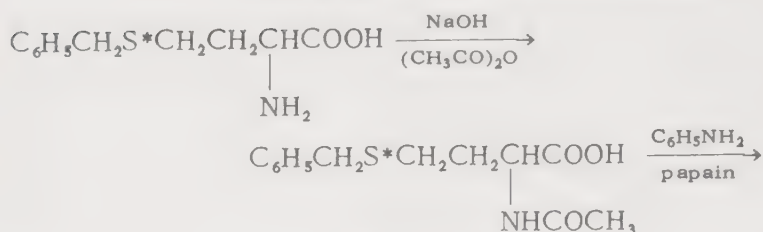
7. The temperature is maintained between 45–50° during this time, and the hydrazoic acid should be 1.5 *N* or greater. The progress of the reaction is followed by the evolution of nitrogen.

8. A drop of the reaction mixture is adjusted to pH 5.5 and chromatographed. If the chromatogram indicates the presence of sulfoxide, additional hydrazoic acid is added.

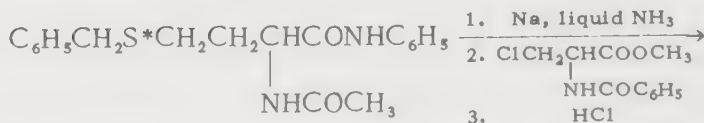
¹G. Toennies and J. J. Kolb, *J. Biol. Chem.*, 128, 399 (1939).

²F. Misani and L. Reiner, *Arch. Biochem.*, 27, 234 (1950).

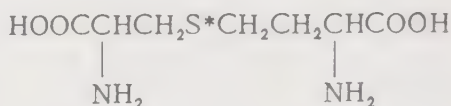
LL-CYSTATHIONINE-S³⁵
(LL-2,7-Diamino-4-thiaoctanedioic-S³⁵)



(a)



(b)



(c)

J. R. Rachele, L. J. Reed, A. R. Kidwai, M. F. Ferger and V. du Vigneaud, J. Biol. Chem., 185, 817 (1950).

A. Procedure

(a) *DL-2-Acetamido-4-(benzylthio)butyric-S³⁵ Acid*, (N-Acetyl-S-benzyl-DL-homocysteine-S³⁵). 2-Amino-4-(benzylthio)butyric-S³⁵ acid, 4.8 g. (0.021 mole), is dissolved in a mixture of 10.6 ml. of 2 N sodium hydroxide and 6.3 ml. of water. The solution is cooled in an ice-bath, and with continuous stirring 50.8 ml. of 2 N sodium hydroxide and 5.08 ml. of acetic anhydride are added during 15 minutes. The mixture is stirred for an additional 30 minutes at room temperature and then is acidified with 22.0 ml. of 6 N sulfuric acid. After the mixture is kept in the refrigerator overnight, it is filtered, and the precipitate is washed with cold 0.2 N hydrochloric acid and then with water. The crude product, 5.36 g., is dissolved in 65 ml. of 15% ethanol and decolorized with activated carbon. After the solution is cooled, 5.07 g. (89%) of crystalline product, m.p. 113.5–114.5° (Note 1), is collected.

(b) *L-2-Acetamido-4-(benzylthio)butyranilide-S³⁵*, (N-Acetyl-S-benzyl-L-homocysteine-S³⁵ Anilide). To 5.07 g. (0.019 mole) of N-acetyl-S-benzyl-DL-homocysteine-S³⁵ in a 250-ml. flask are added 12.9 ml. of 1.08 N sodium hydroxide and 3.44 ml. (0.038 mole) of aniline. Dissolution of the material is effected by the addition of 100 ml. of 0.2 M citrate buffer (pH 5.0) followed by warming of the mixture. The solution is then cooled to room temperature, and 0.286 g. of L-cysteine hydrochloride, dissolved in 10 ml. of buffer, is added. A solution of papain (Note 2) in 14 ml. of

water is introduced, followed by additional buffer to make the total 145 ml. The flask is stoppered, shaken vigorously and stored at 40° for 3 days. The needle-like crystals of the product are collected and washed with water. The compound is dissolved in hot acetone, the solution is filtered, and the solvent is evaporated. The yield of white solid, m.p. 152.5°, is 2.97 g.

(c) *LL-Cystathionine-S³⁵*, (*LL-2,7-Diamino-4-thiaoctanedioc-S³⁵ Acid*). In a tube fitted with a bubbler for the introduction of dry nitrogen and cooled with a Dry Ice-cellosolve bath, 2.07 g. (6.05 mmoles) of *N*-acetyl-*S*-benzyl-*L*-homocysteine-*S³⁵* anilide is added to 30 ml. of liquid ammonia (Note 3). While a slow stream of nitrogen is passed through the mixture, sodium wire is added in small portions until a blue color persists for several minutes. A total of 291 mg. (12.6 mmoles) of sodium is used. The cooling bath is removed, and passage of nitrogen through the mixture is continued until all the ammonia has evaporated. Then, 2.92 g. (12.1 mmoles) of *N*-benzoyl-3-chloro-*L*-alanine methyl ester (Note 4) and 10 ml. of absolute ethanol are introduced, and the mixture is heated at 65–75° for 30 minutes (Note 5). The mixture is refluxed with 126 ml. of 6 *N* hydrochloric acid for 12 hours, cooled overnight and filtered. After the precipitate is washed with cold water, the filtrate and washings are combined and concentrated to dryness *in vacuo*. The residue is dissolved in approximately 20 ml. of 1 *N* hydrochloric acid and filtered through a layer of activated carbon. The pH of the filtrate is adjusted to 6.0–6.5 with ammonium hydroxide, and 20 ml. of ethanol is added. The mixture is cooled overnight and filtered, and the precipitate is washed with water, alcohol and ether. The product is redissolved in about 12 ml. of 1 *N* hydrochloric acid and filtered through carbon. The pH of the filtrate is adjusted to 6.5, 10 ml. of ethanol is added, and the mixture is cooled for several hours. The precipitate is collected, washed with water, alcohol and ether, and dried. The yield of impure product (Note 6) is 1.12 g. (83%), $[\alpha]_D^{22} +22.0^\circ$ (1% solution in 1 *N* hydrochloric acid). After a second recrystallization from 1 *N* hydrochloric acid, the yield is 0.94 g. (70%), $[\alpha]_D^{22} +23.4^\circ$ (1% in 1 *N* hydrochloric acid).

B. Notes

1. The melting point is identical with that observed for 2-acetamido-4-(benzylthio)butyric acid.¹ All melting points are corrected micro melting points.

2. The solution of papain is obtained by extracting 1.44 g. of dried papaya latex with 14. ml. of water.

3. The liquid ammonia is distilled from sodium.

4. *N*-Benzoyl-3-chloro-L-alanine methyl ester was prepared by Rachele, *et al.*, essentially according to the procedure of Karrer² for obtaining the D-isomer.

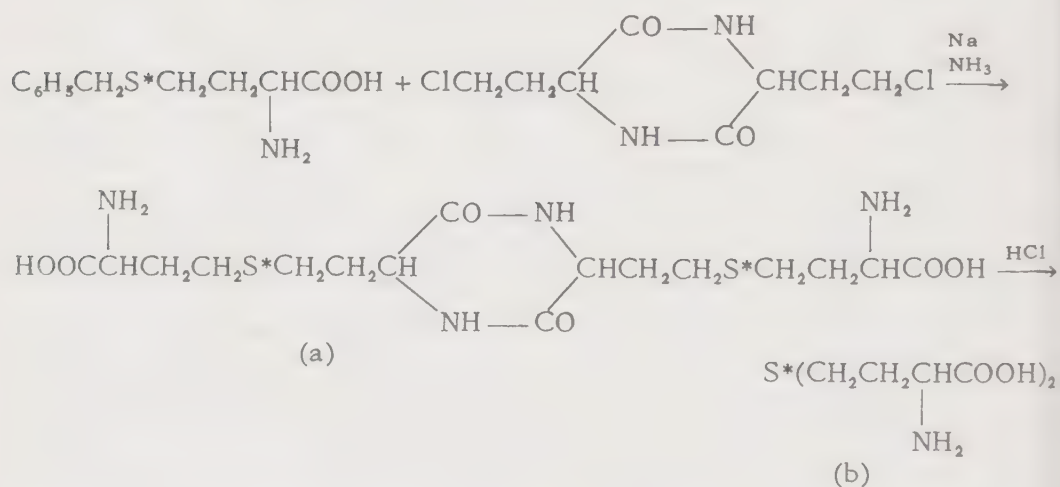
5. A nitroprusside test on a small sample of the reaction mixture was negative.

6. The product gave a positive test for disulfide with sodium nitroprusside after being treated with a 5% solution of sodium cyanide. Following the subsequent recrystallization the test for disulfide was negative.

¹L. J. Reed, A. R. Kidwai and V. du Vigneaud, *J. Biol. Chem.*, **180**, 571 (1949).

²R. Karrer, K. Escher and R. Widmer, *Helv. Chim. Acta*, **9**, 301 (1926).

4,4'THIOBIS(2-AMINO BUTYRIC)-S³⁵ ACID
(Homolanthionine-S³⁵)



J. A. Stekol and K. Weiss, *J. Biol. Chem.*, **179**, 67 (1949).

A. Procedure (Note 1)

(a) 3,6-Bis[2-(3-amino-3-carboxypropylthio)ethyl]-2,5-piperazinedione-S³⁵. To a solution of 5 g. of sodium in 250 ml. of liquid ammonia is added 24 g. (0.1 mole) of 2-amino-4-(benzylthio)butyric-S³⁵ acid, in small portions, with constant agitation of the reaction mixture (Note 2). Then, 12 g. (0.05 mole) of 3,6-bis(2-chloroethyl)-2,5-piperazinedione is added in portions with thorough mixing. After all of the diketopiperazine compound is added, the ammonia is allowed to evaporate, and the reaction vessel is then evacuated. The ammonia-free residue is dissolved in about 150 ml. of ice-cold water, and the solution is filtered through a bed of Norit. The pH of the solution is adjusted to 6 with hydrochloric acid. After 10-12 hours in the refrigerator, the precipitate is collected, washed with cold

water and then recrystallized from dilute ammonium hydroxide solution by the addition of hydrochloric acid to pH 6. The crystalline product is recrystallized from dilute ethanol and then collected and washed successively with ethanol and ether; decomposition point, 270–273°.

(b) 4,4'-Thiobis(2-aminobutyric)-S³⁵ Acid, (Homolanthionine-S³⁵). To 150 ml. of 20% hydrochloric acid is added 10 g. of the above diketopiperazine derivative, and the mixture is refluxed for 3 hours. After recrystallization from dilute ethanol and drying in *vacuo* over phosphorus pentoxide, the yield of homolanthionine-S³⁵, which decomposes at 269–272°, is 9.3 g. (Note 3).

B. Notes

1. This synthesis of homolanthionine-S³⁵ is according to Stekol's¹ adaptation of the procedure of Snyder, *et al.*^{2,3} for the synthesis of methionine and similar thio ethers.

2. The blue color due to excess sodium is discharged by adding 2-amino-4-(benzylthio)butyric acid in small amounts.

3. Since, in the synthesis described, racemic 2-amino-4-(benzylthio)butyric acid and 3,6-bis(2-chloroethyl)-2,5-piperazinedione were used, the resulting product is probably a mixture of *racemic*- and *meso*-forms of homolanthionine.

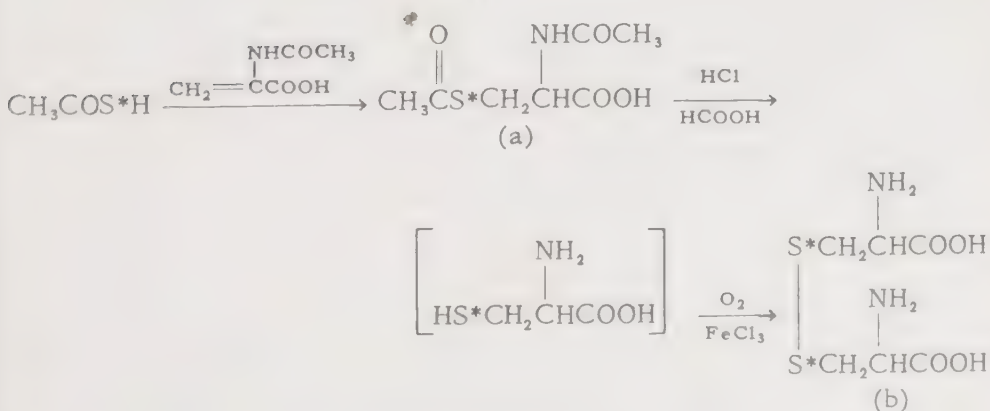
¹J. A. Stekol, J. Biol. Chem., 173, 153 (1948).

²H. R. Snyder, J. H. Andreen, G. W. Cannon and C. F. Peters, J. Am. Chem. Soc., 64, 2082 (1942).

³H. R. Snyder and G. W. Cannon, *ibid.*, 66, 511 (1944).

CYSTINE-S₂³⁵ (3,3'-Dithiodialanine-S₂³⁵)

METHOD I



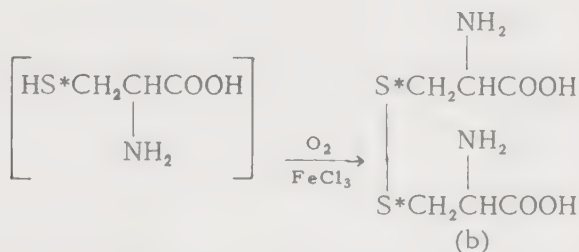
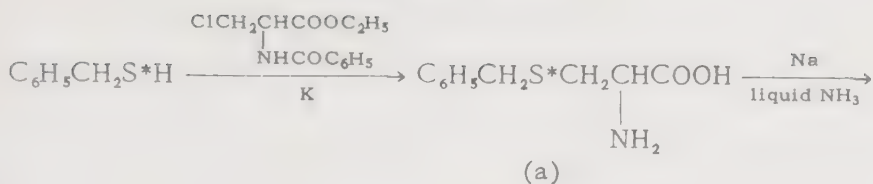
H. R. V. Arnstein and P. T. Grant, Biochem. J., 57, 360 (1954).

A. Procedure

(a) *N,S-Diacetylcysteine-S³⁵*, (*2-Acetamido-4-thio-5-oxohexanoic-S³⁵ Acid*). Approximately 1.52 g. (20 mmoles) of thioacetic-S³⁵ acid is vacuum-distilled into a Carius tube, which contains 5.18 g. (40 mmoles) of 2-acetamidoacrylic acid¹, and is cooled in liquid air. The tube is sealed and heated at 100° for 2 hours and 120° for 1.5 hours. At the end of the reaction any volatile material is removed by vacuum distillation at 100° into a trap cooled in liquid air. The residual solid product is repeatedly extracted with dry, boiling chloroform (8 to 25-ml. portions). The chloroform extract is removed with a filter stick and collected in a receiver, to which two alkaline permanganate traps are attached for absorbing volatile isotopic material. The combined chloroform extracts are evaporated with an air-stream to 100 ml. (Note 1).

(b) *Cystine-S₂³⁵*, (*3,3'-Dithiodialanine-S₂³⁵*). The remaining 95 ml. of the above chloroform solution is evaporated to 30 ml., and 10 ml. of light petroleum is added slowly. After 72 hours, the mother liquor is removed with a filter stick, and the product is dried with an air-stream. The dry product is heated under reflux for 3 hours with 40 ml. of a mixture of 4 *N* hydrochloric and formic acids (1:1 by volume). The acid is then removed by repeated evaporation *in vacuo* at 100°, and the solid residue is dissolved in water (about 30 ml.). After the pH of the solution is adjusted to 8 with concentrated ammonium hydroxide and 2 drops of 1% (w/v) aqueous ferric chloride are added, a slow stream of air is passed through the solution for 8 hours. Acetic acid is then added to pH 4.5 and, after 72 hours at 4°, the pale-buff solid is collected. The product is dissolved in 10 ml. of 2 *N* hydrochloric acid and treated with carbon. The filtrate is then adjusted to pH 4.5 with 6 *N* ammonium hydroxide and 3 *N* sodium acetate. The white precipitate of cystine-S₂³⁵ is centrifuged after 72 hours at 4° and washed twice with 5-ml. portions of water, twice with 10-ml. amounts of ethanol and finally with 20 ml. of ether; yield, 0.98 g. (Note 2).

METHOD II



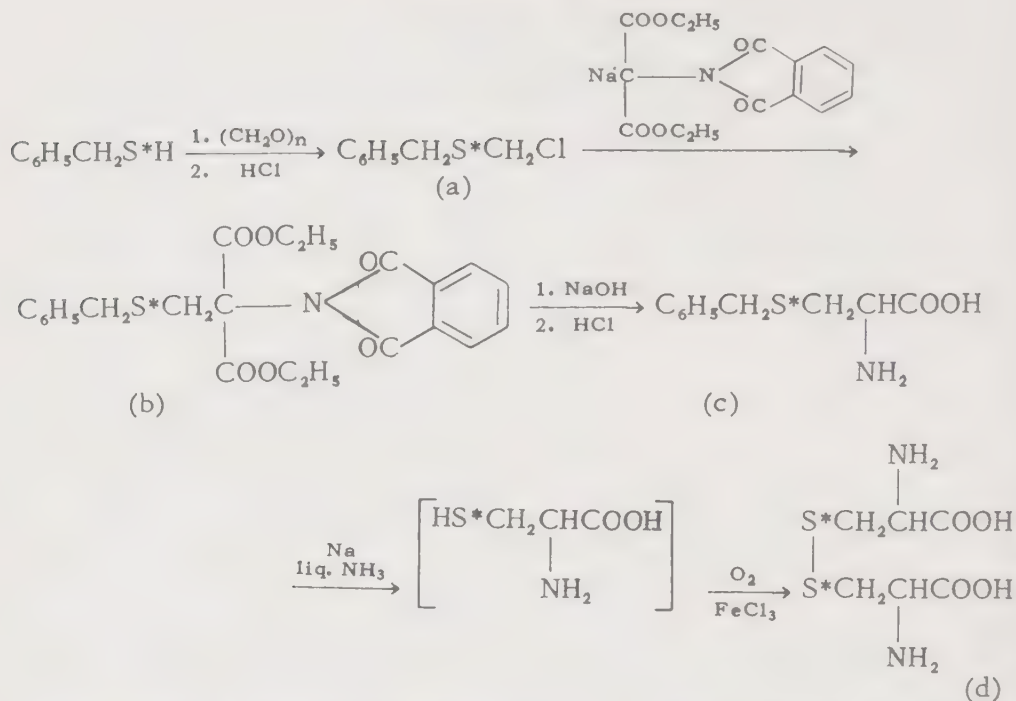
J. B. Melchior and H. Tarver, Arch. Biochem., 12, 301 (1947).

A. Procedure

(a) *3-(Benzylthio)alanine-S³⁵* (*S*-Benzylcysteine-S³⁵). A mixture of ethyl 2-benzamido-3-chloropropionate (10 mmoles) (Note 3), α -toluenethiol-S³⁵ (5.35 mmoles) and potassium metal (6 mmoles) in 6 ml. of absolute ethyl alcohol is heated under reflux at 65–75° for 30 minutes. After the addition of 125 ml. of 6 *N* hydrochloric acid, the reaction mixture is refluxed for 19 hours. The clear hydrolysate is cooled in an ice-bath, and benzoic acid is collected and washed with several small portions of ice-water. The filtrate and washings are evaporated to dryness *in vacuo*; the residue is dissolved in water, and *S*-benzylcysteine-S³⁵ is precipitated by adjusting the pH to 6 with ammonium hydroxide. After the mixture is chilled, the product is collected, washed with several portions of alcohol and ether, and dried. The yield is 0.90 g. (80%).

(b) *Cystine-S₂³⁵*, (*3,3'*-Dithiodialanine-S₂³⁵). The above *S*-benzylcysteine-S³⁵ is converted to cystine-S₂³⁵ according to the procedure of Wood and du Vigneaud (see Method III, Note 4).

METHOD III



A. M. Seligman, A. M. Rutenburg and H. Banks, J. Clin. Invest., 22, 275 (1943).

A. Procedure (Note 4)

(a) *Benzyl Chloromethyl Sulfide-S³⁵*. A mixture of 6.3 g. of α -toluene-thiol-S³⁵ and 2.1 g. of polyoxymethylene is cooled in an ice-bath and saturated with dry hydrogen chloride. Then, 2.5 g. of calcium chloride is added, and the mixture is kept at room temperature for 24 hours. The solid material is removed by filtration and washed with dry ether. The combined filtrate and washings are then distilled. After removal of ether, the fraction boiling at 102° (2 mm.) is collected (Note 5). The yield is 6 g. (68%).

(b) *Ethyl α -(Benzylthio)methyl-1,3-dioxo-2-isoindolinemalonate-S³⁵*. A mixture of 6 g. of benzyl chloromethyl sulfide-S³⁵, 14 g. of ethyl 1,3-dioxo-2-sodium-2-isoindolinemalonate and 30 ml. of toluene is refluxed for 2.5 hours. The precipitated sodium chloride is filtered from the solution and washed with toluene. Removal of the toluene *in vacuo* leaves a viscous oil (Note 6).

(c) *3-(Benzylthio)alanine-S³⁵, (S-Benzylcysteine-S³⁵)*. The oily residue of malonic ester derivative, above, is suspended in 77 ml. of 50% ethanol; 6 ml. of dioxane and 2 drops of phenolphthalein indicator are added, and the mixture is heated to 50°. With stirring, 8.8 ml. of 5 N sodium hydroxide is added at such a rate that the temperature is maintained at 55–60°. When all the alkali is added, the temperature of the

solution is raised to 70°. The solution is stirred for 15 minutes while the temperature falls spontaneously; then enough hydrochloric acid is added to make the mixture acidic to the indicator. The volume of the solution is reduced 50% by distillation under vacuum. Water is added to make the volume 120 ml., and 14.4 ml. of concentrated hydrochloric acid is added (Note 7). The solution is heated for 1.5 hours, 72 ml. of concentrated hydrochloric acid is added, and heating is continued for 2 hours. The solution is concentrated to dryness, the residue is taken up with water, and the solution is evaporated again. The residue is dissolved in 48 ml. of water and made neutral to Congo red with ammonium hydroxide. After the precipitate is collected and washed with water, it is suspended in boiling 95% ethanol and filtered while hot. This extraction is repeated several times until the phthalic acid is dissolved, leaving *S*-benzylcysteine- S^{35} as a crystalline residue (Note 8) which weighs 3.1 g. (42%) (Note 9).

(d) *Cystine-S₂³⁵*, (3,3'-*Dithiodialanine-S₂³⁵*). *S*-Benzylcysteine- S^{35} , 1.25 g., is added to 21 ml. of liquid ammonia in portions, and metallic sodium, in small pieces, is added as fast as it can react. When a permanent blue color of dissolved sodium remains for 15 minutes, ammonium chloride is added until the excess sodium is destroyed. The ammonia is then allowed to evaporate, and the residue is taken up in ice-water. After the aqueous solution is extracted with ether, concentrated hydrochloric acid is added until the highly alkaline solution is just alkaline to phenolphthalein indicator. Without isolation of the cysteine- S^{35} , 2 drops of ferric chloride solution is added, and air is bubbled through the solution until the nitroprusside test for the sulphhydryl group is negative. The solution is neutralized to litmus with hydrochloric acid and kept overnight. The precipitate is collected, washed with water and then dissolved in hot 1 *N* hydrochloric acid. The solution is treated with carbon, filtered, cooled, and neutralized with ammonium hydroxide. The precipitate of cystine- S_2^{35} , which is cooled and washed with water, alcohol and ether, weighs 0.53 g. (75%).

B. Notes

1. A sample of *N,S*-diacetylcysteine- S^{35} was prepared for isotopic assay by dilution of 5 ml. of this solution with light petroleum (b.p. 60–80°). After 48 hours at 0°, the crystalline product was collected and recrystallized from ethyl acetate; yield, 169.1 mg. (79.7%). The sample was diluted with nonisotopic material and recrystallized to constant activity.

2. Arnstein and Grant present a procedure for the resolution of DL-cystine- S^{35} via the brucine salt and purification of the D-isomer through repeated dilution of the L-form with nonisotopic carrier.

3. Ethyl 2-amino-3-chloropropionate hydrochloride was prepared from serine ethyl ester by the method of Fischer and Raske² and benzoylated by the method of Karrer.³

4. The following procedure is an adaptation of the method of Wood and du Vigneaud⁴ for the synthesis of cystine.

5. A crystalline residue, b.p. about 200°, weighed 1.9 g. and was probably bis(benzylthio)methane-S₂³⁵. The same product was obtained in high yield when sodium α -toluenethiolate was treated with an excess of methylene chloride.

6. Wood and du Vigneaud⁴ crystallized this residue from hot absolute ethanol; after recrystallization, the yield of product, m.p. 81-82°, was 70%.

7. Upon acidification of the solution, carbon dioxide was evolved.

8. After recrystallization from dilute hydrochloric acid solution by the addition of ammonium hydroxide, the melting point of halogen-free S-benzylcysteine was 215-216°.³

9. The derivative, *N*-acetyl-3-(benzylthio)alanine-S³⁵, m.p. 156.5-157.5°, has been prepared by Wood⁵ using an adaptation of the acetylation procedure of du Vigneaud and Irish,⁶ which employs acetic anhydride in a basic medium.

C. Other Preparations

S-Benzylcysteine-S³⁵, m.p. 213-214°, has been prepared,⁵ in 38-44% yield, from benzylthiomagnesium-S³⁵ chloride, obtained from benzylmagnesium chloride and sulfur-S³⁵, and 2-amino-3-chloropropionic acid hydrochloride.

A sample of S-benzyl-DL-cysteine-S³⁵ has been resolved⁵ by the isotopic dilution method described by Wood and Gutmann⁷ to obtain S-benzyl-L-cysteine-S³⁵. The latter compound was converted to L-cystine-S₂³⁵, in 77% yield, by the method of Wood and du Vigneaud.⁴ (See cystine-S₂³⁵, Method III, Note 4).

Two syntheses of cystine, which are designed for the use of isotopic sulfur, have been described by Fry.⁸ Both are dependent upon the initial transformation of serine into 2-phenyl-2-oxazoline-4-carboxylic acid. In one procedure, the hydrochloride salt of the oxazoline is rearranged to methyl 2-benzamido-3-chloropropionate, and the chlorine is replaced directly by the sulfhydryl group. The resultant *N*-benzoylcysteine methyl ester is hydrolyzed and oxidized to obtain a 58% yield of cystine. In the second procedure, the thiobenzoic acid salt of 2-phenyl-2-oxazoline-4-carboxylic acid is rearranged to *N,S*-dibenzoylcysteine, which is converted to optically active cystine in 42% yield. Both yields are based on sulfur and do not take recovered sulfur into account.

Arnstein, *et al.*,⁹ have also prepared *N,N'*-bis(phenylacetyl)-L-cystine S₂³⁵ by the acylation of L-cystine-S₂³⁵ with phenylacetyl chloride.

- ¹M. Bergmann and K. Grafe, Hoppe-Seyler's Z. physiol. Chem., 187, 191 (1930).
- ²E. Fischer and K. Raske, Ber., 40, 3717 (1904).
- ³R. Karrer, K. Escher and R. Widmer, Helv. Chim. Acta, 9, 301 (1926).
- ⁴J. L. Wood and V. du Vigneaud, J. Biol. Chem., 131, 267 (1939).
- ⁵J. L. Wood and L. Van Middleworth, *ibid.*, 179, 529 (1949).
- ⁶V. du Vigneaud and O. J. Irish, *ibid.*, 122, 349 (1937-1938).
- ⁷J. L. Wood and H. R. Gutmann, *ibid.*, 179, 535 (1949).
- ⁸E. M. Fry, J. Org. Chem., 15, 438 (1950).
- ⁹H. R. V. Arnstein, M. Clubb and P. T. Grant, *Proceedings Radioisotope Conference*, Volume I, Academic Press Inc., Publishers, New York, 1954, p. 306.

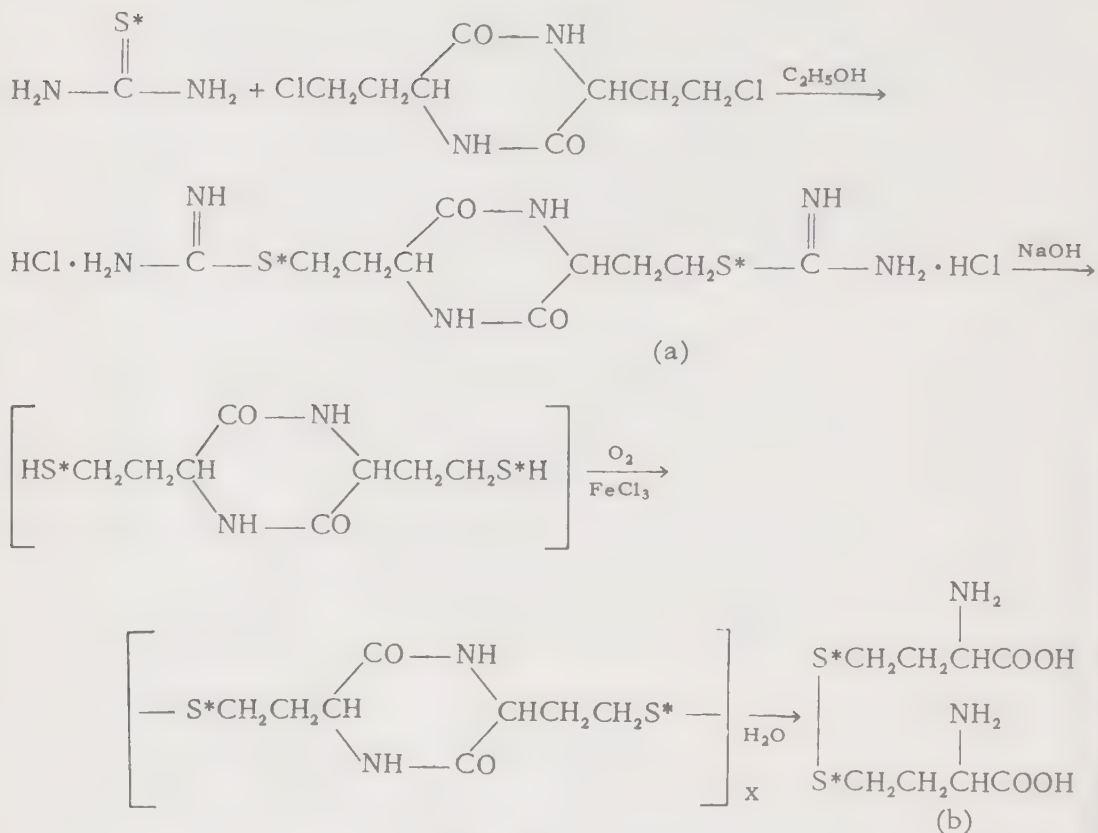
HOMOCYSTINE-S₂³⁵
[4,4'-Dithiobis(2-aminobutyric Acid)-S₂³⁵]

$$\begin{array}{c} \text{C}_6\text{H}_5\text{CH}_2\text{S}^*\text{CH}_2\text{CH}_2\underset{\text{NH}_2}{\text{CH}}\text{COOH} \xrightarrow[\text{C}_4\text{H}_9\text{OH}]{\text{Na}} \left[\text{HS}^*\text{CH}_2\text{CH}_2\underset{\text{NH}_2}{\text{CH}}\text{COOH} \right] \xrightarrow[\text{FeCl}_3]{\text{O}_2} \\ \text{NH}_2 \\ | \\ \text{S}^*\text{CH}_2\text{CH}_2\text{CHCOOH} \\ | \qquad \qquad | \\ \text{NH}_2 \qquad \qquad \text{NH}_2 \\ | \qquad \qquad | \\ \text{S}^*\text{CH}_2\text{CH}_2\text{CHCOOH} \end{array}$$

A. M. Seligman, A. M. Rutenburg and H. Banks, *J. Clin. Invest.*, 22, 275 (1943).

2-Amino-4-(benzylthio)butyric-S³⁵ acid, 1.56 g., is suspended in 30 ml. of butyl alcohol. The mixture is refluxed, and 1.6 g. of sodium is added in small pieces, during 2 1/2 hours (Note 1). The solution is cooled and extracted with water (Note 2). The aqueous extract is neutralized with hydrochloric acid and then made faintly alkaline with ammonium hydroxide. A crystal of ferric chloride is added, and oxygen is bubbled through the solution until the initial dark color disappears. The solution is neutralized and concentrated *in vacuo* to about 75 ml. After several hours, the precipitate is collected and washed with water, alcohol and ether. Concentration of the mother liquor yields additional product. The total yield of homocystine-S₂³⁵ is 0.6 g. (73%).

METHOD II



J. A. Stekol and K. Weiss, J. Biol. Chem., 185, 577 (1950).

A. Procedure

Homocystine-S³⁵ is prepared from thiourea-S³⁵ according to an adaptation of the procedure of Snyder,¹ which follows.

(a) *S,S'-(2,5-Dioxo-3,6-piperazinediethyl)diisothiuronium-S₂³⁵ Chloride*. A mixture of 47.8 g. (0.2 mole) of 3,6-bis(2-chloroethyl)-2,5-piperazinedione² and 33.5 g. (0.44 mole) of thiourea in 500 ml. of absolute ethanol is refluxed for 24 hours. The reaction mixture is cooled in an ice-bath, and the product, which is collected and air-dried, weighs 77 g. (98%). After recrystallization from water, the white solid darkens at 250° and melts at 255° with decomposition.

(b) *Homocystine-S₂³⁵, [4,4'-Dithiobis(2-aminobutyric Acid)-S₂³⁵]*. A solution of 8 g. (0.2 mole) of sodium hydroxide in 40 ml. of water is added dropwise to a stirred mixture of 19.5 g. (0.05 mole) of the above isothiuronium salt and 100 ml. of water at room temperature. After it has stirred for a total of 1 hour, two crystals of ferric chloride are added, and air is bubbled through the mixture for 48 hours. The mixture is concentrated to dryness under reduced pressure. The residue is dissolved in

250 ml. of concentrated hydrochloric acid, and this solution is refluxed for 3 hours. It is then concentrated to dryness under reduced pressure, and the residue is extracted with several portions of boiling absolute ethanol. The combined alcoholic extracts are treated with carbon and then with an excess of pyridine. The solution is stored in a refrigerator overnight, and the crude product, which weighs 10 g. (74.5%), is collected by filtration. After several recrystallizations from water, the product darkens at 250° and decomposes without melting at 258–263° (Note 3).

B. Notes

1. This procedure is a modification of the synthesis of homocystine reported by Patterson and du Vigneaud.³ The more convenient reduction of 2-amino-4-(benzylthio)butyric acid with sodium and butyl alcohol gave yields of homocystine comparable to those obtained with sodium and liquid ammonia.

2. The sodium salt of homocysteine-S³⁵ was extracted, but the free homocysteine-S³⁵, formed upon acidification of the solution, was not isolated.

3. The value reported in the literature⁴ is 260–265°.

¹H. R. Snyder and G. W. Cannon, J. Am. Chem. Soc., 66, 511 (1944).

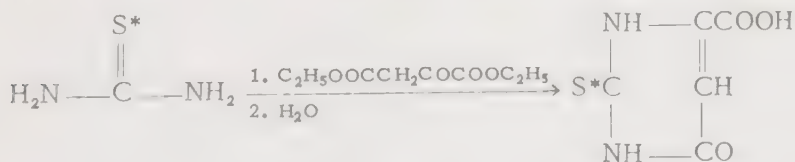
²H. R. Snyder, J. H. Andreen, G. W. Cannon and C. F. Peters, J. Am. Chem. Soc., 64, 2082 (1942).

³W. I. Patterson and V. du Vigneaud, J. Biol. Chem., 11, 393 (1935).

⁴L. W. Butz and V. du Vigneaud, *ibid.*, 99, 135 (1932–1933).

2-THIOÖROTIC-S³⁵ ACID

(1,2,3,6-Tetrahydro-6-oxo-2-thioxo-4-pyrimidinecarboxylic-S³⁵ Acid)



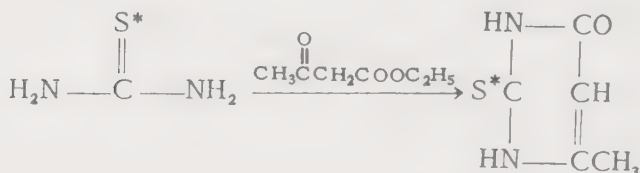
M. J. E. Ernsting and W. Th. Nauta, Rec. trav. chim., 72, 527 (1953).

Procedure

A solution of 6.3 g. of ethyl oxalacetate and 2.53 g. of thiourea-S³⁵ in 66 ml. of absolute alcohol is prepared by heating the mixture. This solution is cooled in a water-bath, and a solution of 1.53 g. of sodium in 66 ml. of acetone is added dropwise. A yellow precipitate forms, and the mixture is heated to boiling in a water-bath. The mixture is kept at room temperature for about 12 hours, 4 g. of sodium hydroxide in 50% alcohol is added, and the mixture is refluxed for 45 minutes. The alcohol is dis-

tilled off, finally under vacuum, while water is simultaneously added. The aqueous solution is acidified with concentrated hydrochloric acid, and the precipitate is collected, washed with water and dried. The yield of 2-thioörotic-S³⁵ acid, which decomposes above 300°, is 3.92 g. (68.5%).

6-METHYL-2-THIOURACIL-S³⁵
[2-Mercapto-6-methyl-4(3*H*)-pyrimidinone-S³⁵]



J. J. Bezem, F. Brunnekreeft, M. J. E. Ernsting, J. Lever and W. Th. Nauta, *Acta Endocrinol.*, 3, 151 (1949).

A. Procedure

6-Methyl-2-thiouracil-S³⁵ is prepared according to the method of Wheeler and McFarland,¹ as in the following example. A mixture of 30 g. of acetoacetic ester, 17.5 g. of thiourea and a solution of 10.6 g. of sodium in 200 ml. of absolute ethanol is warmed for one-half hour. Evaporation of the alcohol on a steam-bath appears to bring the reaction to completion, and the sodium salt of 6-methyl-2-thiouracil remains. A solution of the salt in water is acidified with acetic acid. The precipitate of 6-methyl-2-thiouracil (Note 1) is collected, suspended in warm, dilute acetic acid, filtered, washed with water and dried (Note 2).

B. Notes

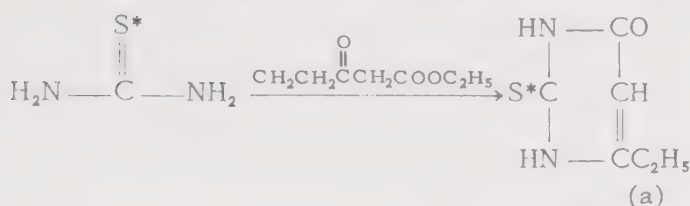
1. 6-Methyl-2-thiouracil melts above 300°.²
2. Ernsting and Nauta³ have prepared 6-methyl-2-thiouracil-S³⁵, in nearly quantitative yield, by essentially the above method.

¹H. L. Wheeler and D. F. McFarland, *Am. Chem. J.*, 42, 101 (1909).

²G. W. Anderson, I. F. Halverstadt, W. H. Miller and R. O. Roblin, Jr., *J. Am. Chem. Soc.*, 67, 2197 (1945).

³M. J. E. Ernsting and W. Th. Nauta, *Landbouwkundig Tijdschr.*, 61, 903 (1949); through *Chem. Abstracts*, 44, 3444 (1950).

6-ETHYL-2-THIOURACIL-S³⁵
[6-Ethyl-2-thio-4(1H,3H)-pyrimidinone-S³⁵]



J. Bell and K. A. MacDonald, J. Chem. Soc., 1951, 1930.

A. Procedure (Note 1)

(a) *6-Ethyl-2-thiouracil-S³⁵*, [*6-Ethyl-2-mercapto-4(3H)-pyrimidinone-S³⁵*]. A mixture of 0.695 g. (9 mmoles) of thiourea-S³⁵, 1.32 g. of ethyl 3-oxovalerate (Note 2) and 0.213 g. of sodium dissolved in 4.6 ml. of anhydrous ethanol is refluxed for 5 hours. The solution is evaporated to dryness, and the residue is dissolved in 9 ml. of hot water. To this solution is added 1.28 ml. of concentrated hydrochloric acid and 0.9 ml. of acetic acid. The precipitate of 6-ethyl-2-thiouracil-S³⁵ is dried at 120° and recrystallized from alcohol; yield 0.98 g. (68.6%), m.p. 228°.

(b) *6-Propyl-2-thiouracil-S³⁵*, [*2-Mercapto-6-propyl-4(3H)-pyrimidinone-S³⁵*]. Condensation of 0.6 g. (8 mmoles) of thiourea-S³⁵ and 1.25 g. of ethyl 3-oxohexanoate (Note 2), according to the above procedure, affords 0.96 g. (71.3%) of 6-propyl-2-thiouracil-S³⁵, m.p. 218°, after recrystallization from alcohol.

B. Notes

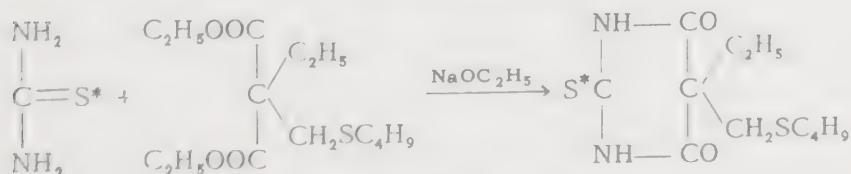
1. The condensation of thiourea-S³⁵ with keto-esters is according to an adaptation of the method described by Robinson and Tomlinson¹ and by Anderson, *et al.*²

2. Improved procedures for the synthesis of ethyl 3-oxovalerate and ethyl 3-oxohexanoate are given by Anderson, *et al.*²

¹R. Robinson and M. L. Tomlinson, J. Chem. Soc., 1935, 1283.

²G. W. Anderson, I. F. Halverstadt, W. H. Miller and R. O. Roblin, Jr., J. Am. Chem. Soc., 67, 2197 (1945).

5-(BUTYLTHIO)METHYL-5-ETHYL-2-THIOBARBITURIC-S³⁵ ACID
(S³⁵-Thionarcon)



E. Bua, A. Cestari and A. Fava, Ricerca Sci., 22, 1932 (1952).

A. Procedure (Note 1)

The condensation of ethyl (butylthiomethyl)ethylmalonate with thiourea-S³⁵ in the presence of sodium ethoxide is according to the method indicated by Bezzi.¹ In lieu of further details regarding the isotopic preparation, the following example is taken from the work of Walter.² A mixture of 0.2 mole of ethyl (butylthiomethyl)ethylmalonate (Note 2), 0.24 mole of thiourea and 0.42 mole of sodium, dissolved in 150 ml. of absolute ethanol, is refluxed for 12 to 18 hours. The alcohol is removed *in vacuo* on a water-bath, and the residue is dissolved in 150–200 ml. of water. The resulting solution is extracted with ether and then acidified with acetic acid. The crude product is extracted into ether, concentrated partially, filtered (Note 3) and evaporated to dryness. The product is then crystallized from alcohol or alcohol-water mixture, m.p. 106.5–107.5° (uncor.).

B. Notes

1. Unsuccessful attempts were made to exchange sulfur-S³⁵ from sodium sulfide-S³⁵ with unlabeled thionarcon.

2. General procedures are given by Walter² for the preparation of alkyl chloromethyl sulfides and (alkylthiomethyl)alkylmalonic esters.

3. Filtration of a concentrated ether solution removed a jelly-like impurity. In case the free acid can not be easily purified, it may be purified as the sodium salt by recrystallization from absolute alcohol. The sodium salts of thiobarbituric acids crystallize readily from absolute alcohol as solvates containing two molecules of alcohol.

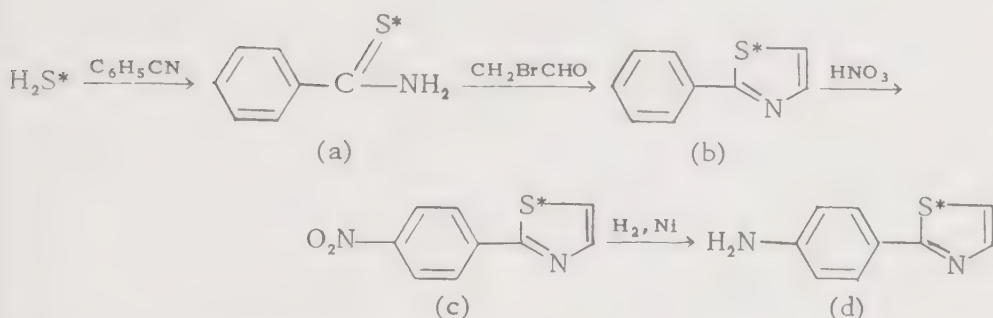
C. Other Preparations

The preparation of sodium thiopental-S³⁵, [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric-S³⁵ acid], in 62% yield based on thiourea-S³⁵, has been reported.³ Starting with thiourea-S³⁵, this group has prepared *S*-(2-diethylaminoethyl)isothiuronium-S³⁵ chloride (100% yield), 2-diethylaminoethanethiol-S³⁵ (71% yield), and 2-(diethylamino)ethyldiphenylthiolacetic-S³⁵ acid (80% yield). From potassium thiocyanate-S³⁵, they have also prepared 4-isopropylbenzaldehyde thiosemicarbazone-S³⁵ (65% yield) and 4'-formylacetanilide thiosemicarbazone-S³⁵ (70% yield). Experimental details were not given for any of the above preparations.

¹S. Bezzi, A. Cestari and A. Cocco, *Farmaco*, 3, 269 (1948).

²L. A. Walter, L. H. Goodson and R. J. Fosbinder, *J. Am. Chem. Soc.*, 67, 655 (1945).

³Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, *Doklady Akad. Nauk S.S.S.R.*, 91, 1129 (1953).

2-(4-AMINOPHENYL)THIAZOLE-S³⁵

H. Noll, E. Sorkin and H. Erlenmeyer, *Helv. Chim. Acta*, 32, 1209 (1949).

A. Procedure

(a) *Thiobenzamide-S³⁵*. According to the procedure of Gabriel and Heymann,¹ a mixture of 20 g. of benzonitrile, 60 ml. of alcoholic ammonia and 60 ml. of alcohol is saturated with hydrogen sulfide and heated in a stoppered flask for 1 hour on a water-bath. After evaporation of the alcohol, the product is recrystallized from water, and the yield is nearly quantitative, m.p. 116° (Note 1).

(b) *2-Phenylthiazole-S³⁵*. According to the following procedure of Erlenmeyer, *et al.*,² 50 g. of depolymerized bromoacetaldehyde and 3 drops of piperidine are added to a solution of 60 g. of thiobenzamide in 180 ml. of absolute alcohol. The mixture is refluxed on a water-bath for 10 hours. The reaction mixture is then freed of alcohol and made alkaline with 2 N sodium carbonate solution. The oily product is steam-distilled and then extracted from the distillate with ether. The ether solution is washed with water and dried over sodium sulfate. After evaporation of the ether, distillation of the residual oil yields 47 g. (72%) of 2-phenylthiazole, b.p. 135–138° (18 mm.).

(c) *2-(4-Nitrophenyl)thiazole-S³⁵*. Nitration of the phenylthiazole is according to the procedure of Adams and co-workers.³ To 10 ml. of concentrated sulfuric acid cooled in an ice-salt bath, 2.5 g. of 2-phenylthiazole is added, with vigorous stirring, during one minute. A previously cooled mixture of 4 ml. of fuming nitric acid (sp. gr. 1.5) and 6 ml. of concentrated sulfuric acid is added dropwise (rapidly). The mixture is stirred for 20 seconds longer and poured into a mixture of 200 g. of cracked ice and 85 ml. of 20% aqueous sodium hydroxide. The precipitate is collected and crystallized from ethyl alcohol-water (Note 2).

(d) *2-(4-Aminophenyl)thiazole-S³⁵* (Note 3). A suspension of 4.2 g. of 2-(4-nitrophenyl)thiazole in 200 ml. of alcohol is hydrogenated over Raney-nickel catalyst at 20°. When the absorption of hydrogen is com-

plete, the solution is filtered and concentrated. The addition of water causes precipitation of the product, which is recrystallized from alcohol-water. The yield of 2-(4-aminophenyl)thiazole is 2.75 g. (75%), m.p. 123-124°.

B. Notes

1. In the isotopic synthesis, Noll, Sorkin and Erlenmeyer obtained a 70% yield of the thiobenzamide-S³⁵.

2. Erlenmeyer, *et al.*,² obtained an 80% yield of 2-(4-nitrophenyl)thiazole, m.p. 147-148°, by this method.

3. The nitro compound is reduced catalytically according to the procedure of Erlenmeyer.² The reduction can also be done with iron and dilute hydrochloric acid.³

C. Other Preparations

N¹-2-Thiazolyl-S³⁵-sulfanilamide (S₁³⁵-sulfathiazole) has been prepared,⁴ starting with thiourea-S³⁵, *via* the following intermediates: 2-aminothiazole-S³⁵ and N⁴-acetyl-N¹-2-thiazolyl-S³⁵-sulfanilamide.

N¹-(4-Methyl-2-thiazolyl)sulfanilamide-S³⁵ (S₁³⁵-sulfamethiazole) has been prepared⁵ from sulfuric-S³⁵ acid *via* the following intermediates: N-acetylsulfanilic-S³⁵ acid (75% yield), N-acetylsulfanilyl-S³⁵ chloride (76% yield) and N⁴-acetyl-N¹-(4-methyl-2-thiazolyl)sulfanilamide-S³⁵ (40% yield). The yield in the final step was also 40%.

N¹-(4-methyl-2-thiazolyl-S³⁵)sulfanilamide has also been prepared⁵ starting with thiourea-S³⁵. The intermediates were: 2-amino-4-methylthiazole-S³⁵ (82% yield) and N⁴-acetyl-N¹-(4-methyl-2-thiazolyl-S³⁵)sulfanilamide (63%). Hydrolysis of the latter compound gave the final product in 40% yield.

Experimental details were not included in either of the above references.

¹S. Gabriel and P. Heymann, *Ber.*, 23, 157 (1890).

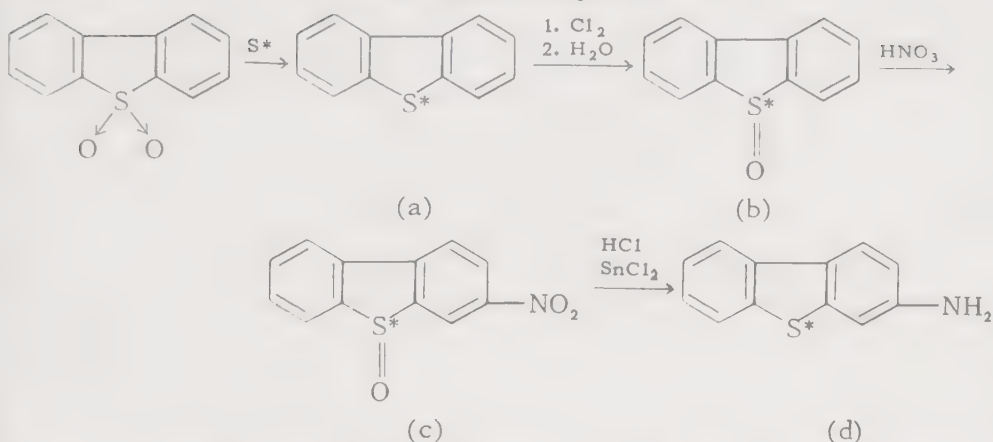
²H. Erlenmeyer, C. Becker, E. Sorkin, H. Bloch and E. Suter, *Helv. Chim. Acta*, 30, 2058 (1947).

³B. S. Friedman, M. Sparks and R. Adams, *J. Am. Chem. Soc.*, 59, 2262 (1937).

⁴H. Noll, J. Bang, E. Sorkin and H. Erlenmeyer, *Helv. Chim. Acta*, 34, 340 (1951).

⁵Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, *Doklady Akad. Nauk S.S.S.R.*, 91, 1129 (1953).

3-DIBENZOTHIOPHENAMINE-S³⁵
(3-Aminodibenzothiophene-S³⁵)



R. R. Brown, S. Kirkwood, L. Marion, S. Naldrett, R. K. Brown and R. B. Sandin, *J. Am. Chem. Soc.*, 73, 465 (1951).

A. Procedure

(a) *Dibenzothiophene-S³⁵* (Note 1). The sulfur-S³⁵ from 0.2076 g. of barium sulfate-S³⁵ and 0.2645 g. of sodium sulfide, added as carrier (Note 2), is dissolved in carbon disulfide. This solution is added to the reaction flask containing 2.57 g. of dibenzothiophene 5,5-dioxide and evaporated to dryness. An atmosphere of dry nitrogen is supplied to the flask, and the evolved sulfur dioxide is swept with a stream of nitrogen through two traps, each containing 3 ml. of 2 N sodium hydroxide. The exchange reaction is effected by heating the solid mixture for 2 hours at 320–330°, 30 minutes at 330–340°, 30 minutes at 360° and finally for 15 minutes at 370–390°. The reaction mixture is cooled and steam-distilled. The cold distillate is extracted with ether. After drying the extract over sodium sulfate and removal of the ether, the residue is twice distilled under vacuum and crystallized from ethanol to obtain 0.2446 g. (31%) of dibenzothiophene-S³⁵, m.p. 98–99°.

(b) *Dibenzothiophene-S³⁵ 5-Oxide* (Note 3). According to the procedure of Fries and Vogt,¹ a solution of 15 g. of dibenzothiophene in 150 ml. of carbon tetrachloride is treated at 0.5° with chlorine until 6 g. of the latter is absorbed. The solution becomes red, and the addition compound which is produced is hydrolyzed by vigorously shaking the reaction mixture with ice and water. The solid is collected, washed with water and dried. The yield of crude dibenzothiophene 5-oxide, m.p. 174–180°, is 15.8 g. (97%). After recrystallization from benzene the yield of pure product is 12.5 g. (77%); m.p. 185–187°.

(c) *3-Nitrodibenzothiophene-S³⁵ 5-Oxide*. The nitration of dibenzothiophene 5-oxide is done according to the procedure of Gilman² for nitration

of the corresponding dioxide. To an ice-cold mixture of 15 g. of dibenzothiophene 5-oxide, 33 ml. of glacial acetic acid and 33 ml. of concentrated sulfuric acid is added, with stirring, 36 ml. of fuming nitric acid (sp. gr. 1.5) during 15 minutes. After the resulting clear solution remains at 0-5° for 30 minutes, it is poured into 200 g. of cracked ice. The gummy solid soon hardens and is collected and washed with water. After the crude material, which weighs 16.5 g. (87%) and melts at 201-205°, is recrystallized from ethanol, the pure 3-nitrodibenzothiophene 5-oxide weighs 14 g. (76%) and melts at 209.5-210.5°.

(d) 3-Dibenzothiophenamine-S³⁵, (3-Aminodibenzothiophene-S³⁵). To a solution of 10 g. of 3-nitrodibenzothiophene 5-oxide in 100 ml. of glacial acetic acid is added a solution of 51 g. of stannous chloride dihydrate in 65 ml. of concentrated hydrochloric acid. A solid product is formed in the exothermic reaction. After remaining at room temperature for 12 hours, the solid is collected and washed with a mixture of equal parts of glacial acetic and concentrated hydrochloric acids. The hydrochloride salt is treated with dilute sodium hydroxide solution, and the free amine is collected, washed with water and dried. The weight of crude material melting at 113-117°, is 8.1 g. (99%). After crystallization from dilute ethyl alcohol, the product, m.p. 121-122.5°, weighs 6.1 g. (75%).

B. Notes

1. The conversion of dibenzothiophene 5,5-dioxide to benzothiophene by means of sulfur has been reported.^{3,4} There are two possible reaction mechanisms: (a) sulfur removes oxygen atoms from the sulfone group, or (b) sulfur displaces the sulfone group as sulfur dioxide. The latter mechanism is highly probable since the product was quite active and the by-products, sulfur dioxide and a small amount of hydrogen sulfide, which were recovered as barium salts, showed activity of less than 4% of that in the original sulfur-S³⁵.

2. The reduction to free sulfur was according to the procedure of Wood.⁵

3. The following steps in the preparation of 3-dibenzothiophenamine-S³⁵ are as described by Brown and co-workers.⁶

C. Other Preparations

The preparation of thiophene-S³⁵ has been reported by Zel'venskii.⁷

¹K. Fries and W. Vogt, *Ann.*, 381, 341 (1911).

²H. Gilman, A. L. Jacoby and H. A. Pacenitz, *J. Org. Chem.*, 3, 108 (1939).

³N. M. Cullinone and C. G. Davies, *Rec. trav. chim.*, 55, 881 (1936).

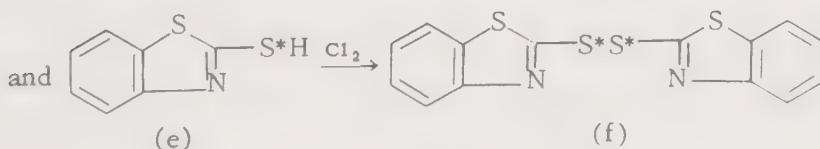
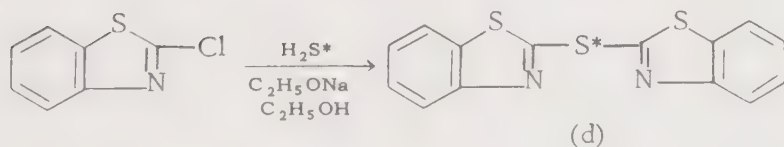
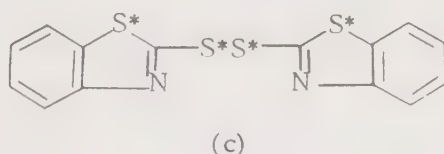
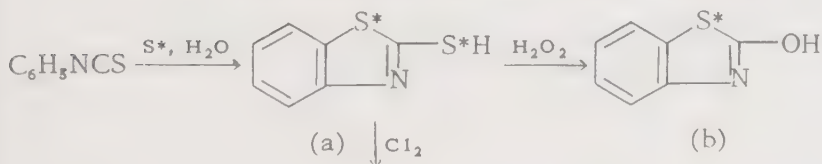
⁴H. Gilman and A. L. Jacoby, *J. Org. Chem.*, 3, 108 (1939).

⁵J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, *J. Am. Chem. Soc.*, 70, 2547 (1948).

⁶R. K. Brown, R. G. Christiansen and R. B. Sandin, *ibid.*, 70, 1748 (1948).

⁷Ya. D. Zel'venskii and V. A. Shalygin, *Neftyanoe Koz.*, 33, No. 8, 65 (1955); through Chem. Abstracts, 49, 15292 (1955).

2,2'-DITHIO-S₂³⁵-BISBENZOTHAZOLE
[2,2'-Bis(benzothiazolyl) Disulfide-S₂³⁵]



E. N. Gur'yanova and M. Ya. Kaplunov., *Doklady Akad. Nauk S.S.S.R.*, 94, 53 (1954); through Chem. Abstracts, 49, 3946 (1955).

A. Procedure

(a) *2-Mercaptobenzothiazole-S₂³⁵*. A mixture of 5 g. of phenyl isothiocyanate, 1.2 g. of sulfur-S³⁵ and 2-3 drops of water is heated in a sealed tube at 250-260° for 3 hours. The yield of 2-mercaptobenzothiazole-S₂³⁵, m.p. 181°, is 80-85%.

(b) *2-Benzothiazolol-S³⁵*. Oxidation of 2-mercaptobenzothiazole-S₂³⁵ with hydrogen peroxide in 1 N potassium hydroxide gives 2-benzothiazolol-S³⁵, m.p. 135-135.5° (Note 1).

(c) *2,2'-Dithiobis(benzothiazole)-S₄³⁵*. Oxidation of 2-mercaptobenzothiazole-S₂³⁵ with chlorine gives this quadruply labeled disulfide.

(d) *2,2'-Thio-S³⁵-bisbenzothiazole*. Hydrogen sulfide-S³⁵ is dissolved in a solution of sodium ethylate in ethanol. Treatment of the resulting solution with 2-chlorobenzothiazole for 10-15 hours at room temperature gives 2,2'-thio-S³⁵-bisbenzothiazole, m.p. 106° and 2-mercapto-S³⁵-benzothiazole.

(e) 2-Mercapto- S^{35} -benzothiazole. The yield of 2-mercapto- S^{35} -benzothiazole from the above reaction is 60-65% (Note 2).

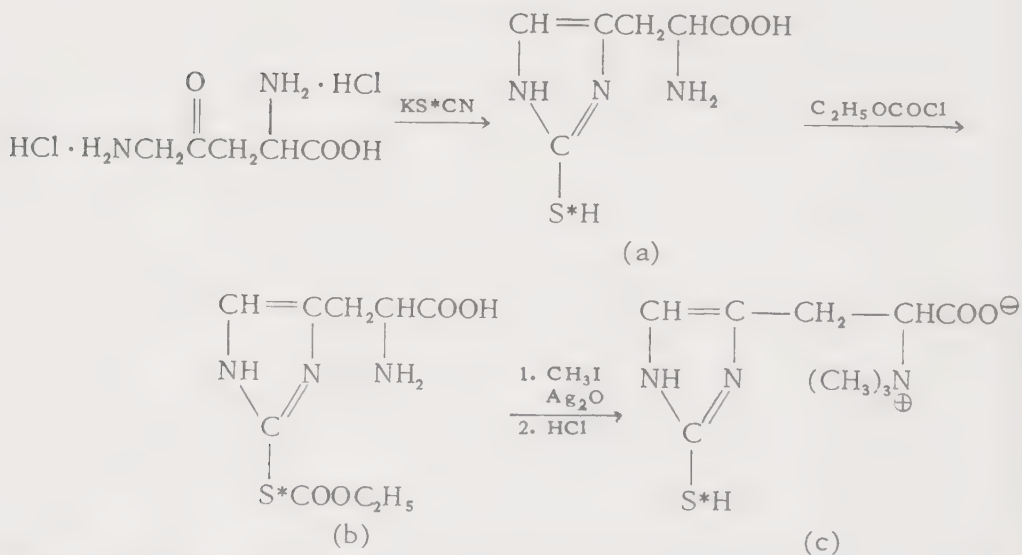
(f) 2,2'-Dithio- S_2^{35} -bisbenzothiazole. Oxidation of 2-mercapto- S^{35} -benzothiazole with chlorine gives this disulfide, m.p. 179-180°.

B. Notes

1. The specific activities of the two products, 2-hydroxybenzothiazole- S^{35} and potassium sulfate- S^{35} , were identical; thus both sulfur atoms in the original 2-mercaptobenzothiazole- S_2^{35} were equally active, indicating that exchange of sulfur took place at 250-260°.

2. Oxidation with hydrogen peroxide, as in (a), gave nonactive 2-benzothiazolol.

THIONEINE- S^{35}



H. Heath, C. Rimington, T. Glover, T. Mann and E. Leone, *Biochem. J.*, 54, 606 (1953); *ibid.*, 54, 689 (1953).

A. Procedure (Note 1)

(a) 2-Mercaptobistidine- S^{35} . A solution of 4-oxoörnithine dihydrochloride, prepared from 65 g. of methyl N^2, N^5 -dibenzoyl-4-oxoörnithine methyl ester (Note 2), is heated on a boiling water-bath. To this solution are added four 10-g. portions of potassium thiocyanate at one half-hour intervals. After heating for 3 hours, the solution is treated with carbon, filtered and concentrated under reduced pressure to about 75 ml. On adjustment of the pH of the solution to 5, with sodium carbonate, crystallization begins and, after storage at 4°, the nearly colorless product is

collected, washed with water and dried. The yield of 2-mercaptohistidine is 19.3 g. (58%). It decomposes at 300° without melting.

(b) 2-(Carbethoxythio)histidine- S^{35} Dihydrochloride. To a suspension of 4.6 g. of 2-mercaptohistidine in 100 ml. of ethanol is slowly added 5 ml. of ethyl chlorocarbonate. This mixture is refluxed on a boiling water-bath until dissolution is complete. The solution is cooled, 150 ml. of dry ether is added, and crystallization of the product is effected at -10° . The nearly colorless crystalline product is collected, washed with dry ether and dried. The yield of 2-(carbethoxythio)histidine, m.p. 189° (dec.), is 5.8 g. (71%) (Note 3).

(c) Thioneine- S^{35} . To a solution of 3.32 g. (0.01 mole) of 2-(carbethoxythio)histidine dihydrochloride in 20 ml. of water is added a suspension of 12 g. of freshly prepared silver oxide in 50 ml. of water. With agitation and cooling, 1.9 ml. (0.03 mole) of methyl iodide is added to the mixture, which is then shaken mechanically for 1 hour. Then, 50 ml. of concentrated hydrochloric acid is added, and the suspension is centrifuged. The precipitate is washed twice with 25-ml. portions of 5 *N* hydrochloric acid by centrifugation. The combined solution is boiled for 2 hours and concentrated to dryness under reduced pressure. The residue, dissolved in 50 ml. of water, is treated with hydrogen sulfide to remove silver. After the mixture is heated and centrifuged, the supernatant liquid is treated with saturated aqueous phosphotungstic acid until all the thioneine is precipitated. The precipitate of thioneine phosphotungstate is centrifuged, washed with water, suspended in water and made alkaline with saturated barium hydroxide solution. The precipitate of barium phosphotungstate is centrifuged, and the solution is immediately acidified with 2 *N* sulfuric acid. The barium phosphotungstate is extracted twice with water, and the total solution is adjusted to pH 7 with barium hydroxide solution. After centrifugation and carbon treatment, the solution is concentrated under reduced pressure until crystallization occurs. After recrystallization from aqueous ethanol the product is dried *in vacuo* over phosphorus pentoxide at 105° . The yield of thioneine, m.p. 290° , is 0.8 g. (30%); $[\alpha]_D + 47^{\circ}$ (*c*, 1; *l* = 2, in water) (Note 4).

B. Notes

1. Thioneine- S^{35} was prepared according to the following procedure of Heath¹ using 25 g. of potassium thiocyanate- S^{35} .

2. 2-Mercaptohistidine was prepared according to a modification of the procedure of Ashley and Harington,² which was based on the observation of Kossel and Edlbacher³ that benzylation of histidine methyl ester dihydrochloride results in ring opening to yield methyl 2,4,5-tribenzamido-4-pentenoate. The latter compound is hydrolyzed with hydrochloric acid to obtain 4-oxoörrnithine dihydrochloride.

3. 2-(Carboethoxythio)histidine dihydrochloride is very soluble in water and slightly soluble in methanol and ethanol.

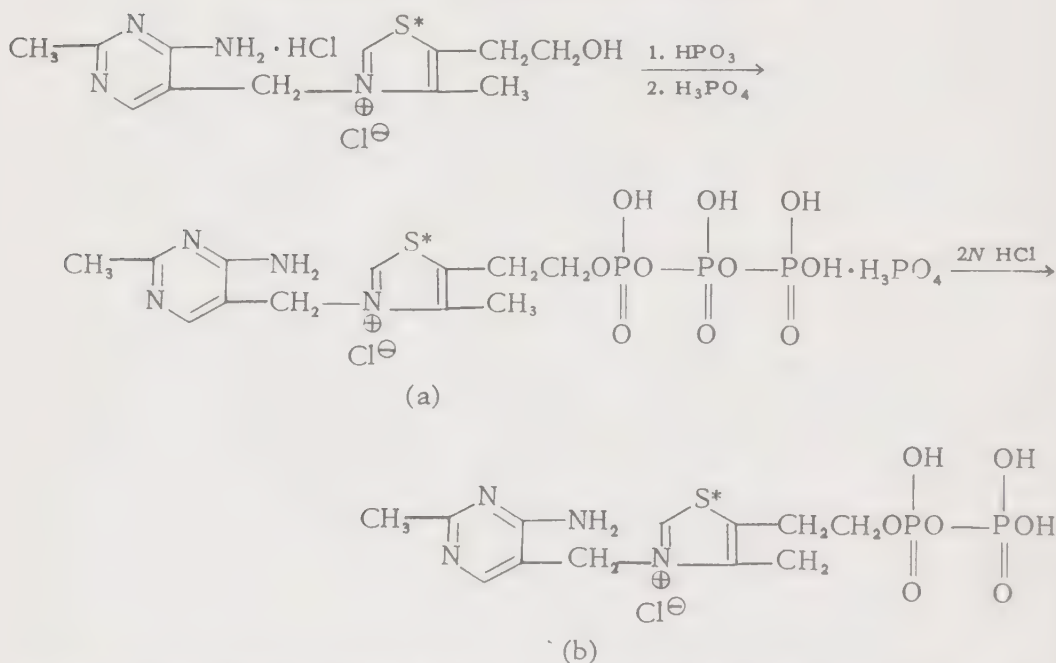
4. The ultraviolet absorption spectrum showed a maximum at 2580 Å., $\epsilon = 16,000$. Synthetic and natural thioneine behaved identically in the following tests: formation of a stable dihydrate; formation of a monohydrochloride, m.p. 250°; paper chromatography, R_f 0.87 in phenol and 0.32 in collidine.

¹H. Heath, A. Lawson and C. Rimington, J. Chem. Soc., 1951, 2215.

²J. N. Ashley and C. R. Harington, *ibid.*, 1930, 2586.

³A. Kossel and S. Edlbacher, Hoppe-Seyler's Z. physiol. Chem., 93, 396 (1915).

COCARBOXYLASE-S³⁵
(Thiamine-S³⁵ Pyrophosphate Chloride)



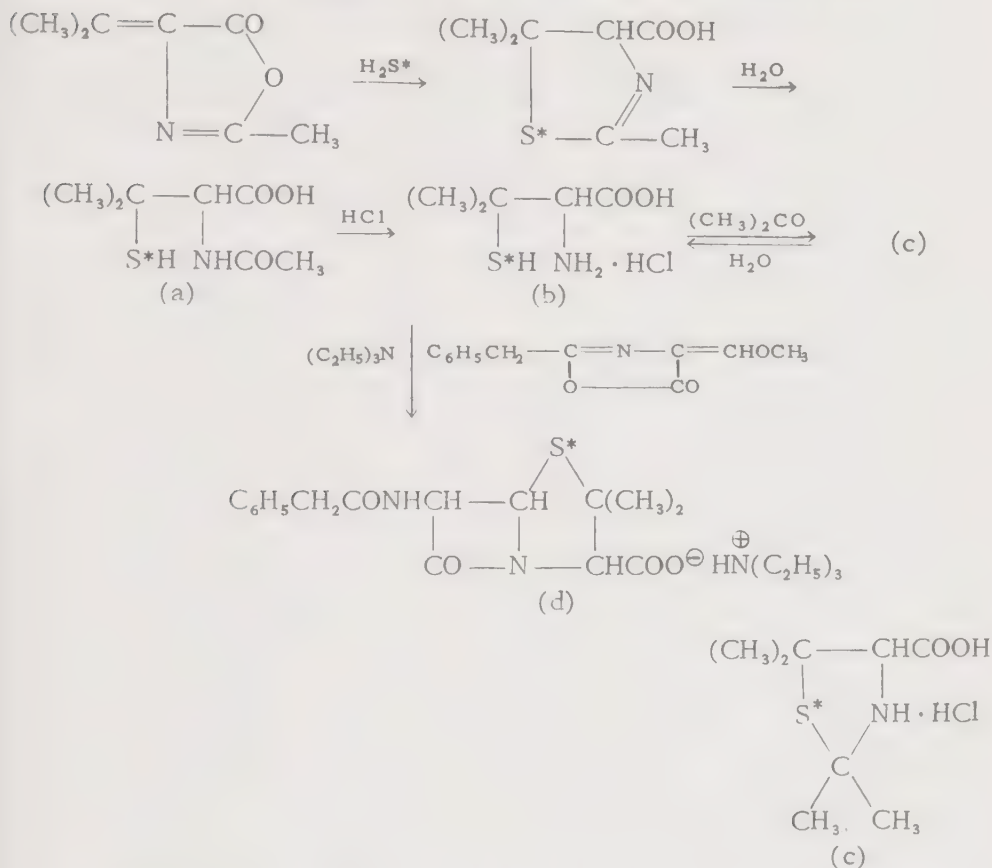
M. Koike, Vitamins (Japan), 6, 420 (1953); through Chem. Abstracts, 48, 5194 (1954).

Procedure

(a) *Thiamine-S³⁵ Triphosphate Phosphate*. A mixture of 200 mg. of thiamine-S³⁵ hydrochloride and 0.8 ml. of metaphosphoric acid is heated at 105° for 15 minutes. The mixture is cooled and diluted with 15 ml. of water containing a few drops of 85% phosphoric acid, and the insoluble material is removed by centrifugation. To the supernatant liquid is added 50 ml. of acetone-ethanol (1:1). The yield of thiamine-S³⁵ triphosphate phosphate is 435 mg. (89%).

(b) *Cocarcboxylase-S³⁵*, (*Thiamine-S³⁵ Pyrophosphate Chloride*). The above triphosphate is hydrolyzed with 2 N hydrochloric acid for 3.5 hours at room temperature. The yield of thiamine-S³⁵ pyrophosphate chloride, precipitated by the addition of acetone-ethanol (1:1), is 60.5%.

TRIETHYLAMMONIUM BENZYL PENICILLINATE-S³⁵



V. du Vigneaud, J. L. Wood and M. E. Wright, *The Chemistry of Penicillin* by H. T. Clarke, J. R. Johnson and R. Robinson, Princeton University Press, Princeton, N. J., 1949, Chapter 23, p. 892.

A. Procedure

(a) *N-Acetyl-3-mercaptopvaline-S³⁵*, (*N-Acetylpenicillamine-S³⁵*). A solution of 250 mg. of freshly prepared 4-isopropylidene-2-methyl-2-oxazolin-5-one in 1 ml. of anhydrous methanol, which is contained in a small flask connected to a gas generator, is cooled in a Dry Ice-bath, and the system is evacuated with an oil-pump. The system is then isolated by means of a stopcock, and the Dry Ice-bath is replaced by an ice-water bath. The

hydrogen sulfide-S³⁵ from 1.99 mmoles of barium sulfide-S³⁵ (Note 1) is allowed to react with the oxazolone as the apparatus is shaken gently. Finally, the gas generator is immersed in a bath at 50–55° for 1 hour, and the reaction mixture is then kept at room temperature for 24 hours. At this time, the methanol solvent is distilled into the gas generator, and the reaction flask is isolated from the generator by means of a stopcock (Note 2). The reaction mixture is heated with 1 ml. of water at 90°, under a nitrogen atmosphere, for 15–20 minutes (Note 3). Decolorizing carbon is added, and the solution is filtered. The product, which crystallizes during this process, is recovered by washing the carbon with ethanol and hot water. The combined filtrate is evaporated to dryness, and 1 ml. of hot water is added to the residue. This solution is cooled to obtain 220 mg. of crystalline *N*-acetylpenicillamine-S³⁵, m.p. 168–174° (capillary), 175–180° (micro).

(b) *3-Mercaptovaline-S³⁵ Hydrochloride*, (*Penicillamine-S³⁵ Hydrochloride*). The above acetylpenicillamine is heated under nitrogen with 3.1 ml. of 2 *N* hydrochloric acid for 6 hours, in an oil-bath at 125°. The resulting solution is extracted twice with ether, and is then evaporated to dryness, *in vacuo*.

(c) *2,2,5,5-Tetramethyl-4-thiazolidinecarboxylic-S³⁵ Acid Hydrochloride*. The dry residue of penicillamine-S³⁵ hydrochloride is dissolved in boiling acetone. The acetone solution is concentrated to 1–2 ml. and cooled. After 2 hours, the yield of crude isopropylidene derivative is 152 mg. The crystals are dissolved in a minimum of methanol containing a trace of hydrogen chloride. Slow addition of acetone to this solution, followed by ether, yields 148 mg. of crystalline product; the m.p. is 157–160° (micro), after the sample is dried at 50° *in vacuo* for 1 hour.

(d) *Triethylammonium Benzylpenicillinate-S³⁵*. To a solution of 113 mg. of penicillamine-S³⁵ hydrochloride in 23 ml. of dry pyridine is added 138 mg. of 2-benzyl-4-methoxymethylene-2-oxazolin-5-one, and the mixture is heated to 110° for 18 minutes (Note 4). The pyridine is evaporated *in vacuo*; a 25-ml. chloroform solution of the residue is cooled in an ice-bath and washed successively with 25 ml. of cold 1 *N* hydrochloric acid, 2 ml. of ice-water and two 5-ml. portions of saturated sodium chloride solution. The cold chloroform solution, dried over magnesium and sodium sulfates, is then evaporated to dryness without warming. The residue is dissolved in about 1 ml. of acetone, and 30 ml. of anhydrous ether is added. The solution is filtered to remove precipitated sludge and diluted to a volume of 50 ml. with ether (Note 5). An ether solution of free benzylpenicillin, containing 273,000–283,000 units of antibiotic activity, is mixed with the synthetic material, and the volume is reduced to about 60 ml. To the ether solution, dried over magnesium sulfate, is added an excess of 10% triethylamine in ether. After the mixture is cooled overnight, the crystalline triethylammonium benzylpenicillinate-S³⁵ is collected

and washed with ether; the yield is 271 mg. The crude product is washed with two portions of dry acetone (5 ml. total), leaving 185 mg. of material which is recrystallized from acetone-ether to obtain 158 mg. of labeled product (Note 6).

B. Notes

1. The hydrogen sulfide-S³⁵ is generated by the addition of 3 ml. of 100% phosphoric acid to the sulfide-S³⁵.

2. Excess hydrogen sulfide-S³⁵ is recovered from the gas generator.

3. The intermediate, 2,5,5-trimethyl-2-thiazoline-4-carboxylic-S³⁵ acid, was hydrolyzed without isolation.

4. A modified, improved synthesis of benzylpenicillin was later reported by du Vigneaud and co-workers.¹ The synthesis was divided into two steps: D-penicillamine hydrochloride and 2-benzyl-4-methoxymethylene-2-oxazolin-5-one were first condensed in pyridine containing triethylamine which gave an insoluble crude product; the latter was treated with pyridinium chloride in hot pyridine to obtain benzylpenicillin in low yield (about 0.1%). Crystalline triethylammonium benzylpenicillinate was isolated from the reaction mixture by the countercurrent distribution principle of Craig.²

5. The total solution had 104-122 Oxford units of penicillin activity by two methods of assay.

6. After further dilution of the triethylamine salt and several recrystallizations, a part of the material was converted to sodium benzylpenicillinate-S³⁵; micro m.p. 214-216°, $[\alpha]_D^{21.5} +289^\circ$ (0.606% in water). Benzylpenicillic-S³⁵ acid was prepared by treating a sample of sodium salt with 0.1 N sodium hydroxide followed by neutralization with 1 N hydrochloric acid, the free acid melted (micro) at 182-184°. Radioactivity determinations on the above two compounds, with corrections for dilutions, were constant within experimental error, thus establishing the identity of the synthetic and authentic compounds.

¹V. du Vigneaud, F. H. Carpenter, R. H. Holley, A. H. Livermore and J. R. Rachele, *The Chemistry of Penicillin*, by H. T. Clarke, J. R. Johnson and R. Robinson, Princeton University Press, Princeton, N. J., 1949, Chapter 28, p. 1018.

²L. C. Craig, *J. Biol. Chem.*, 155, 519 (1944); L. C. Craig, C. Golumbic, H. R. Mighton and E. Titus, *ibid.*, 161, 321 (1945).

Drierite and removal of the toluene at reduced pressure, the product weighs 0.64 g. (69%) (Note 4).

B. Notes

1. The reaction is rapid (about 30 sec.), particularly with 0.2 g. of aluminum chloride.

2. The preparation of this compound is according to the procedure described by Fletcher.¹ A general method of synthesis starting with phosphorus pentasulfide and the appropriate alcohol or phenol, followed by chlorination of the intermediate *O,O*-dialkyl or diaryl phosphorodithioic acid, has been developed by Fletcher and co-workers.²

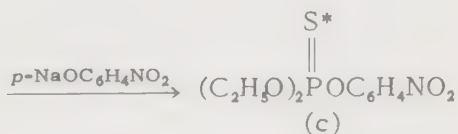
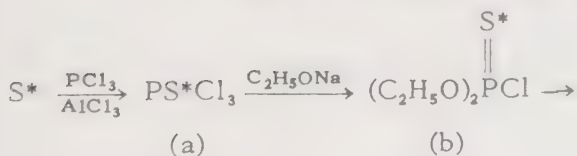
3. Fletcher¹ records the boiling point of 71.5-72° (7 mm.) for ethyl phosphorochloridothionate, n_D^{25} 1.4684.

4. Parathion is a pale-yellow oil, b.p. 157-162° (0.6 mm.), n_D^{25} 1.5370.

¹J. H. Fletcher, J. C. Hamilton, I. Hechenbleikner, E. I. Hoegberg, B. J. Sertl and J. T. Cassaday, *J. Am. Chem. Soc.*, **70**, 3943 (1948).

²*Idem*, 72, 2461 (1950).

PARATHION-S³⁵

(Diethyl *p*-Nitrophenyl Phosphorothionate-S³⁵)

J. A. Jensen and G. W. Pearce, J. Am. Chem. Soc., 74, 3184 (1952).

A. Procedure

(a) *Thiophosphoryl-S³⁵ Chloride* (Note 1). Sulfur-S³⁵ (16 mc.) dissolved in benzene is placed in a 22 × 150-mm. test tube equipped with a standard-taper joint. The solution is evaporated to dryness with a gentle stream of dry air which is introduced through a capillary tube and emerges above the benzene surface. The air escapes through a second capillary; both tubes are mounted in a two-hole rubber stopper. Inactive sulfur is added to make the total 75 mmoles. To this mixture is added 78 mmoles of phosphorus trichloride (4% excess) and 18 mmoles of anhydrous aluminum chloride. The tube is connected to an Allihn condenser, and the contents are heated to boiling on a water-bath. The reaction is

complete in 10 minutes, giving a clear, dark-brown liquid. The crude product is distilled to obtain 74.7 mmoles (99.5%) of thiophosphoryl-S³⁵ chloride, boiling range 123–126°, sp. gr. 1.62 ± 0.01 .

(b) *Ethyl Phosphorochloridothionate-S³⁵* (Note 2). A solution of 74.7 mmoles of thiophosphoryl-S³⁵ chloride in 35 ml. of benzene is placed in a 250-ml. 3-necked flask fitted with stirrer, thermometer and dropping funnel. A solution of 3.63 g. (158 mmoles) of sodium in 100 ml. of absolute ethanol is added evenly during 4 hours, while the stirred reaction mixture is maintained at 5° in an ice-bath. The reaction mixture is stirred for an additional 3 hours following the last addition of sodium ethoxide, and the product is allowed to separate overnight. The flask is then attached to a Lux-Whitmore distillation head. With the pressure reduced to 21 cm., dry air is admitted through a capillary tube, and the benzene and alcohol are distilled into a receiver immersed in a Dry Ice-acetone bath. The remaining concentrated slurry is then treated with 50 ml. of water and 50 ml. of benzene and transferred to a separatory funnel. After separation of the benzene layer, the water layer is extracted with three 10-ml. portions of benzene. The combined benzene solution is washed with three 20-ml. portions of water and dried over Drierite. The benzene solution is evaporated, with a dry-air stream, to a volume of 20 ml. and transferred to a 50-ml. still pot which is then connected to a silvered column equipped with a micro Lux-Whitmore distilling head. With the pressure in the system reduced to 15 cm. (Note 3), the mixture is warmed until the benzene is removed. The pressure is then reduced to 25 mm., and the product is distilled. The yield of ethyl phosphorochloridothionate-S³⁵, b.p. 96–99° (25 mm.), is 12.62 g.; sp. gr. 1.10; n_D^{26} 1.4674.

(c) *Parathion-S³⁵*, (*Diethyl p-Nitrophenyl Phosphorothionate-S³⁵*). Ethyl phosphorochloridothionate-S³⁵, 12.62 g. (67 mmoles), is transferred to a 250-ml. flask, equipped with an Allihn condenser, and 80 ml. of acetone and 10.8 g. (67 mmoles) of sodium *p*-nitrophenoxide are added. The mixture is refluxed for 5 hours on a steam-bath, cooled to room temperature and filtered. The salt is washed with three 10-ml. portions of acetone. Acetone is distilled from the combined filtrate and washings at atmospheric pressure. Vacuum is applied to remove the last traces of acetone, and the residue is washed into a 600-ml. separatory funnel with 50 ml. of benzene. The benzene solution is washed with five 20-ml. portions of 5% sodium carbonate solution and with three 20-ml. portions of water and dried over Drierite. After removal of benzene (Note 4), the product is distilled at 1–2 mm. pressure. An initial 1-ml. fraction distilling up to 152° is discarded. The yield of product, boiling at 152° to 162°, is 12.92 g.; n_D^{25} 1.5380; sp. gr. 1.26 ± 0.01 . The over-all yield is 59.2% (Note 5).

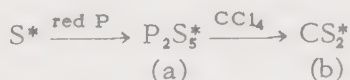
B. Notes

1. The method used was described by Knotz.¹
2. The following procedures are an adaptation of the methods described by Fletcher.^{2,3}
3. A trap cooled with Dry Ice-acetone is inserted between the receiver and the pump.
4. Last traces of benzene were removed at 3-mm. pressure and a temperature of 109°.
5. A small amount of high-boiling residue remained in the still pot. The somewhat high refractive index value of the product was probably due to the presence of small amounts of this high-boiling material. The product was estimated to be 95% parathion.

¹F. Knotz, *Sterr. Chem. Ztg.*, 50, 128 (1949).

²J. H. Fletcher, J. C. Hamilton, I. Hechenbleikner, E. I. Hoegberg, B. J. Sertl and J. T. Cassaday, *J. Am. Chem. Soc.*, 70, 3943 (1948).

³*Idem*, 72, 2461 (1950).

CARBON DISULFIDE-S₂³⁵

Yu. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, *Doklady Akad. Nauk S.S.S.R.*, 91, 1129 (1953).

A. Procedure

(a) *Phosphorus Pentasulfide-S₅³⁵*. A finely ground mixture of 2.5 g. of sulfur-S³⁵ and 1 g. of red phosphorus is fused in a thin-walled test tube, under an atmosphere of carbon dioxide. When the tube has cooled, its upper portion is cut off, and the lower portion containing the phosphorus pentasulfide-S₅³⁵ is ground in a mortar. The yield of product amounts to 3.1 g. (89.4%).

(b) *Carbon Disulfide-S₂³⁵*. A mixture of 2.22 g. of powdered phosphorus pentasulfide-S₅³⁵ and 2.31 g. of carbon tetrachloride is heated in a sealed tube for 7 hours at 300–325° (Note 1). The resulting mixture, consisting of carbon disulfide-S₂³⁵, thiophosphoryl-S³⁵ chloride and carbon tetrachloride, is placed in the flask of a distillation apparatus. With cooling and shaking of the mixture, a solution of 12 g. of potassium hydroxide in 10 ml. of water is added from a dropping funnel. The resulting mixture is carefully heated on a water-bath, and the product begins to distill when the vapor temperature reaches 43–44°; the majority of the product distills at 46–47°, and heating is continued until the vapor temperature reaches 55–57°. The yield of carbon disulfide-S₂³⁵ is 0.9–0.93 g. (77.3%).

B. Notes

1. The viscous reaction mixture becomes a slightly colored mobile fluid.

2. The product contained 4.5-5.6% of carbon tetrachloride. The alkaline residue, which contains potassium sulfide-S³⁵, was treated with 18% hydrochloric acid, and the resulting hydrogen sulfide-S³⁵ was recovered.

C. Other Preparations

The preparation of carbon disulfide-S₂³⁵ by the exchange of sulfide-S³⁵ ion in aqueous solution with carbon disulfide as a separate phase has been described.^{1,2} The reaction was thought to proceed through sulfide exchange with thiocarbonate ion. The latter was decomposed with acid, and the resulting carbon disulfide-S₂³⁵ was recovered by extraction with carbon tetrachloride. Carbon disulfide-S₂³⁵ has also been prepared,³ in 65-70% yields, by the passage of sulfur-S³⁵ vapor over hot charcoal in a quartz tube.

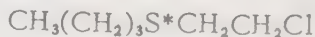
¹C. F. Strittmatter, T. Peters, Jr. and R. W. McKee, Arch. Ind. Hyg. and Occupational Med., 1, 54 (1950).

²R. R. Edwards, F. B. Nesbett and A. K. Soloman, J. Am. Chem. Soc., 70, 1670 (1948).

³K. H. Busing, W. Sonnenschein, E. W. Becker and H. Dreiheller, Z. Naturforsch., 8b, 495 (1953).

BUTYL 2-CHLOROETHYL SULFIDE-S³⁵

(a)



(b)

J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, J. Am. Chem. Soc., 70, 2547 (1948).

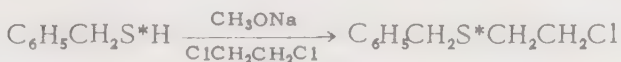
Procedure

(a) 2-(Butylthio)ethanol-S³⁵, (Butyl 2-Hydroxyethyl Sulfide-S³⁵). To a solution of 1-butanethiol-S³⁵ (prepared from 116 mg. of barium sulfate-S³⁵) in petroleum ether are added 1 ml. of water, containing 40 mg. of ethylene chlorohydrin, and 1 ml. of 1 N sodium hydroxide. The mixture is shaken at room temperature for 3 to 4 hours until all the thiol-S³⁵ has reacted. The aqueous layer is separated, and the organic layer is washed with 2 ml. of water. The combined aqueous layers are extracted repeatedly with 1-ml. portions of petroleum ether. The combined petroleum

ether solution is centrifuged and then passed through an 8×50 -mm. column of Permutit (Eimer and Amend No. 901194). The product is then eluted with 25 ml. of dry benzene, and the solution is concentrated at room temperature under reduced pressure. The residue is distilled at 0° and 10^{-3} mm. in a Dry Ice-cooled microsublimator. The distilled product is transferred to a Carius tube with 2 ml. of petroleum ether.

(b) *Butyl 2-Chloroethyl Sulfide-S³⁵*. To the above solution of 2-(butylthio)ethanol-S³⁵ is added 3 ml. of concentrated hydrochloric acid. The tube is sealed and shaken for 24 hours at 65° . The product is isolated in the manner described for the benzyl analog with the exception that the temperature is not raised above -40° at 10^{-3} mm. for removal of the last traces of petroleum ether. The yield of product, usually analytically pure without distillation, is 33-46 mg. (44-62%).

BENZYL 2-CHLOROETHYL SULFIDE-S³⁵



A. M. Seligman, A. M. Rutenburg and H. Banks, J. Clin. Invest., 22, 275 (1943).

A. Procedure

To 6 g. (0.0484 mole) of α -toluenethiol-S³⁵ is added 0.048 mole of sodium methoxide in methanol, with cooling. Then, 50 g. of ethylene chloride is added, and the mixture is kept overnight. After it is heated to the boiling point, the reaction mixture is cooled, acidified and washed with water. The washings are extracted with a small amount of ethylene chloride which is added to the organic layer. The ethylene chloride solution is dried with sodium sulfate, and the solvent is removed at atmospheric pressure. The yield of pale-yellow oil, boiling at 100° to 110° (3 mm.), is 8 g. (90%).

C. Other Preparations

Benzyl 2-chloroethyl sulfide-S³⁵ has been prepared from 2-(benzylthio)ethanol-S³⁵ and concentrated hydrochloric acid¹ and from α -toluenethiol-S³⁵ and ethylene chloride.²

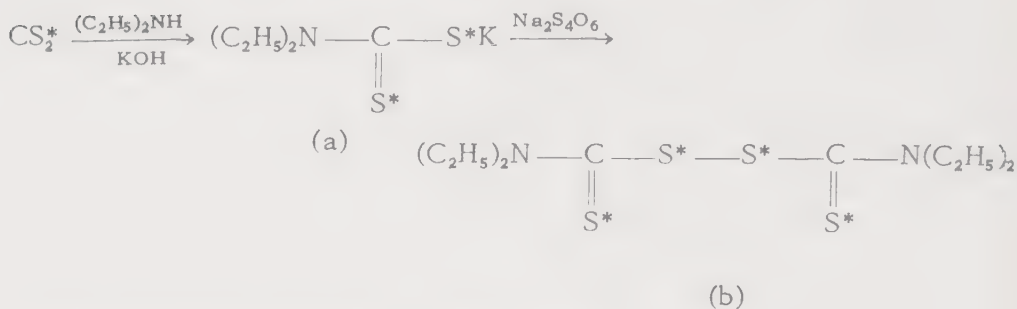
Benzyl 2-chloroethyl-C₁¹³/₂ sulfide-S³⁵ has been prepared,³ using 1,2-dichloroethane-C₁¹³ in the latter reaction.

¹J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, J. Am. Chem. Soc., 70, 2547 (1948).

²H. Tarver and C. L. A. Schmidt, J. Biol. Chem., 130, 67 (1939).

³G. W. Kilmer and V. du Vigneaud, *ibid.*, 154, 247 (1944).

BIS(DIETHYLTHIOCARBAMOYL)-S₂³⁵ DISULFIDE-S₂³⁵
(Tetraethylthiuram-S₂³⁵ Disulfide-S₂³⁵)



L. Eldjarn, Acta Chem. Scand., 3, 644 (1949).

A. Procedure

(a) *Potassium Diethyldithiocarbamate-S₂³⁵*. According to the following procedure described by van Kolmeschate and Stern,¹ 7.3 g. of diethylamine, 10 ml. of ethanol and 7.6 g. of carbon disulfide are carefully mixed in a flask, which is cooled with water if necessary (Note 1). A solution of 5.6 g. of potassium hydroxide, dissolved in a minimum amount of water and diluted with 10 ml. of ethanol, is added dropwise. The mixture is cooled to room temperature and poured into an excess of ether. The white, crystalline product, in suspension, is collected, washed with ether and dried. The yield is approximately 80%. The product is recrystallized from a little alcohol by the addition of ether.

(b) *Bis(diethylthiocarbamoyl)-S₂³⁵ Disulfide-S₂³⁵, (Tetraethylthiuram-S₂³⁵ Disulfide-S₂³⁵)*. The oxidation of the above dithiocarbamate to tetraethylthiuram disulfide is effected with sodium tetrathionate according to the procedure of Flemming and Klein² (Note 2). With stirring and ice-cooling, an aqueous solution of sodium tetrathionate (Na₂S₄O₆) is added during 1 hour to a solution of potassium diethyldithiocarbamate-S₂³⁵. After an additional 15 minutes, the precipitate is washed with water and dried.

B. Notes

1. A solid may separate, but this is of no consequence.
2. The oxidation may also be achieved with iodine according to the procedure of Grodzki.³ A more direct procedure for the preparation of tetraethylthiuram disulfide has been described by Blake.⁴ Bis(diethylamine) sulfide⁵ is added to carbon disulfide. The yield of tetraethylthiuram disulfide, m.p. 69-90°, is 64.8%.

C. Other Preparations

It has been shown⁶ that tetraethylthiuram disulfide and free sulfur-S³⁵ undergo exchange when heated together in a melt at 95-100°. The

amount of exchange did not increase, apparently, after 7 hours.

¹G. J. van Kolmeschate and R. W. Stern, Chem. Weekblad, 45, 733 (1949); through Chem. Abstracts, 44, 3903 (1950).

²W. Flemming and H. Klein, German, 444014 (1927); Chem. Zentr., 1927 II, 636.

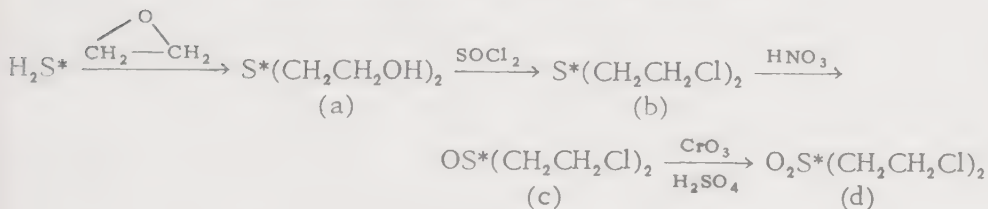
³M. Grodzki, Ber., 14, 2754 (1881).

⁴E. S. Blake, J. Am. Chem. Soc., 65, 1267 (1943).

⁵F. Lengfeld and J. Stieglitz, Ber., 28, 575 (1895).

⁶W. E. Mochel and J. H. Peterson, J. Am. Chem. Soc., 71, 1426 (1949).

BIS(2-CHLOROETHYL) SULFONE-S³⁵



J. C. Bournsnel, G. E. Francis and A. Wormall, Biochem. J., 40, 743 (1946).

A. Procedure

(a) *2,2'-Thiodiethanol-S³⁵*, [*Bis(2-hydroxyethyl) Sulfide-S³⁵*]. Hydrogen sulfide-S³⁵, prepared from barium sulfide-S³⁵, is collected in a trap cooled with liquid air. The all-glass apparatus is then evacuated, and the hydrogen sulfide-S³⁵ is transferred into a reaction flask of known volume, with a side-arm closed by a stopcock and with a manometer attached for measuring the pressure. The hydrogen sulfide-S³⁵ is condensed in the side-arm, and dry ethylene oxide is admitted to the reaction flask to twice the pressure (about 0.5 atmosphere) of hydrogen sulfide-S³⁵. On allowing the hydrogen sulfide-S³⁵ to vaporize into the reaction flask, the reaction proceeds at room temperature, with a quantitative yield of 2,2'-thiodiethanol-S³⁵ within 48 hours.

(b) *Bis(2-chloroethyl) Sulfide-S³⁵*. The 2,2'-thiodiethanol-S³⁵, dissolved in dry chloroform, is treated at 50–60° with a slight excess of thionyl chloride, also in chloroform. The yield of bis(2-chloroethyl) sulfide-S³⁵ is 74% (Note 1).

(c) *Bis(2-chloroethyl) Sulfoxide-S³⁵*. The sulfoxide and sulfone are prepared by adapting the following procedure of Helfrich and Reid.¹ Bis(2-chloroethyl) sulfide, 154 mg., is treated with 308 mg. of concentrated nitric acid. The acid solution is diluted with 1 ml. of water, and after a time the sulfoxide crystallizes. After the crude product is recrystallized from 0.3 ml. of water and dried over phosphorus pentoxide *in vacuo*, the yield of bis(2-chloroethyl) sulfoxide-S³⁵, m.p. 109°, is 74 mg.

(d) *Bis(2-chloroethyl) Sulfone-S³⁵*. According to the procedure described above, 109 mg. of bis(2-chloroethyl) sulfide-S³⁵ is oxidized to the

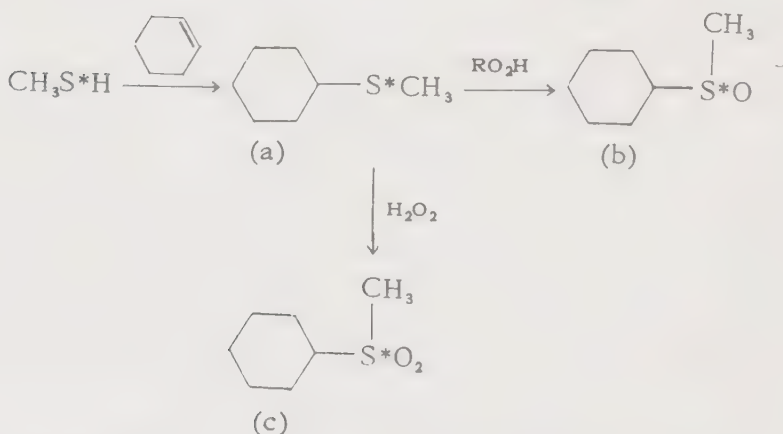
sulfoxide. The excess nitric acid is removed by repeated addition and evaporation of water *in vacuo*. The crude sulfoxide is then oxidized¹ in a hot aqueous solution, containing 5-10% chromic acid and 12% sulfuric acid, for 4 to 6 hours. After dilution with water and cooling of the solution, the product is collected and crystallized from alcohol. The yield of bis(2-chloroethyl) sulfone, m.p. 55°, is 37 mg.

B. Notes

1. Bis(2-chloroethyl) sulfide-S³⁵ was first prepared by passing ethylene into a mixture of sulfur-S³⁵ mono- and dichlorides warmed to 60°. This method was abandoned because of the loss of sulfur-S³⁵ as a by-product. The sulfur-S³⁵ chloride mixture was prepared by passing pure, dry chlorine over barium sulfide-S³⁵ heated in a tube furnace.

¹O. B. Helfrich and E. E. Reid, J. Am. Chem. Soc., 42, 1208 (1920).

CYCLOHEXYL METHYL SULFONE-S³⁵



G. Ayrey, D. Barnard and C. G. Moore, J. Chem. Soc., 1953, 3179.

A. Procedure

(a) *Cyclohexyl Methyl Sulfide-S³⁵*. A mixture of 3.934 g. of cyclohexene (Note 1) and 0.10 ml. of acetone, which is contained in tube A, see Figure XX, 2, is frozen, degassed under vacuum and distilled *in vacuo* into the 50 ml. flask B. With flask B cooled in liquid air, the seal at C is broken and 1.15 g. of methanethiol-S³⁵ is distilled from tube D into the flask which is then sealed off at constriction E. The reaction mixture is irradiated for 16 hours with ultraviolet light and with water cooling.

(b) *Cyclohexyl Methyl Sulfoxide-S³⁵*. The reaction flask is reattached to the vacuum manifold at G. Then, with B cooled in liquid air, the seal at H is broken and a mixture of 2.300 g. of *t*-butyl hydroperoxide (Note 2)

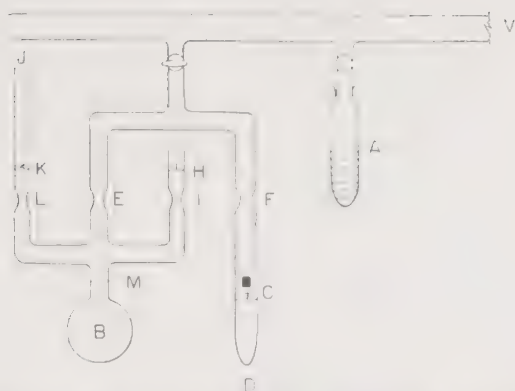


Fig. XX, 2 Apparatus for preparation of cyclohexyl methyl sulfoxide- S^{35} (G. Ayrey, D. Barnard and C. G. Moore). A, tube containing cyclohexene; B, reaction flask; C, H and K, break-seals; D, tube containing methanethiol- S^{35} ; E, F, I and L, constrictions for sealing; V, vacuum manifold.

and 25.0 ml. of absolute methanol is distilled into the reaction flask *in vacuo*. The apparatus is sealed off at constriction I. The reaction mixture is heated at $50.0 \pm 0.1^\circ$ for 64 hours. The flask assembly is re-attached at J to the vacuum manifold to which is already attached a tube with a seal-off constriction. After the apparatus is evacuated, the latter tube is cooled in liquid air, the seal at K is broken and about 10 ml. of low-boiling material is distilled from B into the tube which is then sealed off (Note 3). The flask neck is cut at M and the contents are transferred to a 150-ml. Vigreux distillation unit. Flask B is washed out with a total of 40 g. of cyclohexyl methyl sulfoxide in portions and a little methanol. After removal of low-boiling material, the product is distilled at 10^{-3} mm. After a forerun of 7 g., the yield of cyclohexyl methyl sulfoxide- S^{35} is 35.4 g. (Notes 4 and 5).

(c) *Cyclohexyl Methyl Sulfone- S^{35}* . A solution of cyclohexyl methyl sulfide- S^{35} in a mixture of 2.0 ml. of benzene, 2.0 ml. of petroleum ether and 20 ml. of acetic acid is treated with 2.73 ml. of hydrogen peroxide (8.45 mole/l.). The temperature of the mixture rises to about 40° and is then slowly raised to reflux (Note 6). The mixture is refluxed for 3.0 hours. After removal of solvents and excess of peracetic acid, the residue is distilled in a small molecular-still; the yield of sulfone- S^{35} is 1.07 g. (86%), n_D^{20} 1.4918.

B. Notes

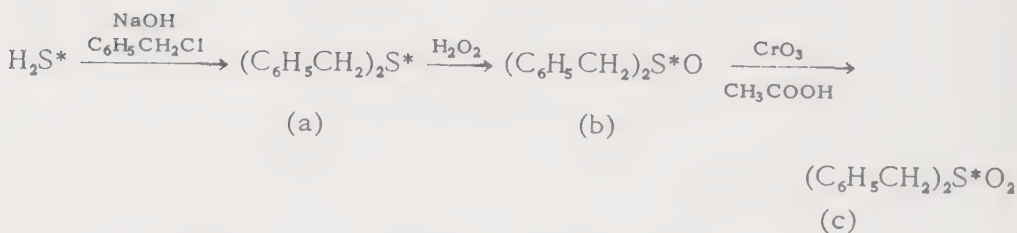
1. The cyclohexene, which was present in 100% excess, was freshly chromatographed on alumina under nitrogen.
2. The previously degassed *t*-butyl hydroperoxide was present in 6% excess based on 100% yield of sulfide.
3. Unchanged methanethiol- S^{35} was recovered in this manner.

4. A similar synthesis with inactive materials gave an 83% yield of cyclohexyl methyl sulfoxide, b.p. 65° (0.01 mm.), n_D^{20} 1.5122. The oxidation of sulfide to sulfoxide was found to be 96.1% complete after 24.0 hours at 50° .

5. A procedure for the removal of cyclohexyl methyl sulfide- S^{35} from the product by means of isotopic dilution and chromatography is described by Ayrey, *et al.*

6. The bath temperature was 130° .

BENZYL SULFONE- S^{35}



F. C. Henriques, Jr. and C. Margnetti, *Ind. Eng. Chem. Anal. Ed.*, **18**, 476 (1946).

A. Procedure (Note 1)

(a) *Benzyl Sulfide- S^{35}* . Using a vacuum manifold, 0.85 mmole of hydrogen sulfide- S^{35} is vacuum-distilled, with liquid nitrogen-cooling, into a tube containing 235 mg. (1.85 mmoles) of benzyl chloride, 75 mg. (1.85 mmoles) of sodium hydroxide, 6 ml. of ethyl alcohol and 1 ml. of water. The tube is then sealed and placed in a steam-bath for 3 days. After this period, the tube is opened, and the contents are extracted 4 times with petroleum ether (b.p. 40 to 60°). The petroleum ether extracts are filtered into a small flask, and the solvent, residual benzyl chloride and benzyl alcohol are distilled off under vacuum. After recrystallization from ethanol, the yield of benzyl sulfide- S^{35} , m.p. 49° , is 168 mg. (92%).

(b) *Benzyl Sulfoxide- S^{35}* . To a solution of 30 g. of benzyl sulfide in 250 ml. of acetone is added 15 g. of 30% hydrogen peroxide. After thorough mixing, the solution is kept at room temperature for 48 hours. The acetone is removed by distillation, and the residual oil solidifies to a white, crystalline solid upon cooling. After recrystallization from petroleum ether, the yield of benzyl sulfoxide, m.p. 132 – 133° , is 22 g. (75%) (Note 2).

(c) *Benzyl Sulfone- S^{35}* . To a solution of 25 g. (0.11 mole) of benzyl sulfide in 130 ml. of glacial acetic acid is added 32 g. of chromic anhydride during a period of one hour. The reaction is started by warming the solution but, once started, it proceeds smoothly. When all the chromic

anhydride has been added, the mixture is refluxed for 15 minutes, and the hot solution is poured onto 500 g. of crushed ice. The precipitate is collected, washed with ice-water and recrystallized three times from 95% alcohol. The yield of benzyl sulfone, m.p. 149.5–150°, is 7 g. (23.7%) (Note 3).

B. Notes

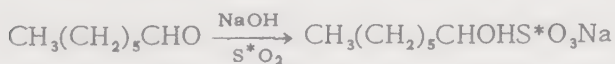
1. The following procedure for the preparation of benzyl sulfide-S³⁵ is an adaptation of the method of Shriner, *et al.*¹ The corresponding sulfoxide and sulfone were prepared according to their methods, as in the following examples.

2. The yield of benzyl sulfoxide-S³⁵, m.p. 134°, was 107 mg. (83%) from 120 mg. of benzyl sulfide-S³⁵.

3. Apparently Henriques and Margnetti oxidized benzyl sulfoxide-S³⁵ to obtain the sulfone-S³⁵. From 60 mg. of benzyl sulfoxide-S³⁵ they obtained 45 mg. (70%) of benzyl sulfone-S³⁵, m.p. 149°.

¹R. L. Shriner, H. C. Struck and W. J. Jorison, *J. Am. Chem. Soc.*, 52, 2060 (1930).

SODIUM 1-HYDROXY-1-HEPTANESULFONATE-S³⁵ (Heptanal Sodium Bisulfite-S³⁵ Addition Product)



H. O. Singher and L. Marinelli, *Science*, 101, 414 (1945).

A. Procedure

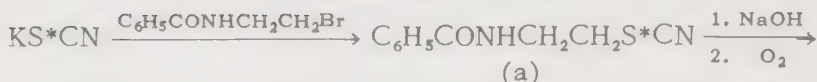
(a) *Sodium 1-Hydroxy-1-heptanesulfonate-S³⁵, (Heptanal Sodium Bisulfite-S³⁵ Addition Product).* Sulfur-S³⁵ dioxide is passed into a slightly alkaline solution of heptanal under an atmosphere of nitrogen (Note 1).

(b) *Sodium 3-Hydroxy-1-phenyl-1-propene-3-sulfonate-S³⁵, (Cinnamaldehyde Sodium Bisulfite-S³⁵ Addition Product).* A solution of this compound is prepared in the same manner as the above (Note 1), from cinnamaldehyde.

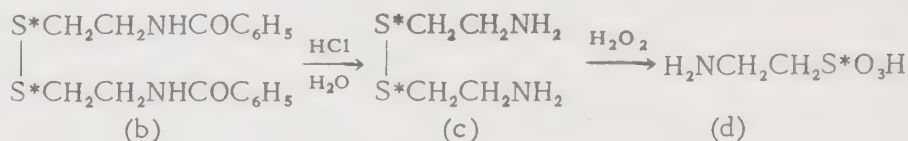
B. Notes

1. These products were not isolated. The aqueous solutions were used directly in amination studies.

TAURINE-S³⁵
(2-Aminoethanesulfonic-S³⁵ Acid)



(a)



(b)

(c)

(d)

L. Eldjarn, Acta Chem. Scand., 5, 677 (1951).

A. Procedure (Note 1)

(a) *2-Benzamidoethyl Thiocyanate-S³⁵*. Potassium thiocyanate-S³⁵ is reacted with *N*-2-bromoethylbenzamide (Note 2) in absolute alcohol at 50°. The average yield of crude product is 90%. After recrystallization from aqueous ethanol, the melting point of the purified product is 80°.

(b) *N,N'-(Dithiodiethylene)dibenzamide-S₂³⁵*, (*Dibenzoylcystamine-S₂³⁵*). *2-Benzamidoethyl thiocyanate-S³⁵* is hydrolyzed with 2 equivalents of potassium hydroxide to obtain *N*-2-mercaptoethylbenzamide-S³⁵. The latter compound is oxidized, without isolation, to *dibenzoylcystamine-S₂³⁵* by bubbling air through the basic alcoholic solution. The yield of *dibenzoylcystamine-S₂³⁵*, m.p. 132.5°, is nearly quantitative.

(c) *2,2'-Dithiobis(ethylamine)-S₂³⁵*, (*Cystamine-S₂³⁵*). *Dibenzoylcystamine-S₂³⁵* is hydrolyzed to *cystamine-S₂³⁵* by heating under reflux with 22% hydrochloric acid.

(d) *Taurine-S³⁵*, (*2-Aminoethanesulfonic-S³⁵ Acid*). *Cystamine-S₂³⁵* is oxidized to *taurine-S³⁵* according to the procedure of Schöberl.¹ Oxidation of the free base with hydrogen peroxide gives a 40% yield of *taurine-S³⁵* (Note 3).

B. Notes

1. This synthesis, which was developed for semi-micro work with sulfur-S³⁵, was described in a preliminary report, and isotopic sulfur was not employed.

2. This compound is prepared by benzoylating the hydrobromide of 2-bromoethylamine in dry pyridine.

3. Oxidation of cystamine hydrochloride with hydrogen peroxide results in most of the sulfur being converted to sulfuric acid.

C. Other Preparations

Taurine-S³⁵ has been synthesized² by a modification of the method of Cortese;³ 2-bromoethylamine hydrobromide was reacted with sodium

sulfite-S³⁵ in a ratio of 1.5:1. The crystalline product melted at 304-307°.

Ethyl ethanesulfonate-S³⁵, b.p. 205-207°, n_D^{20} 1.1497, d_{20} 1.1427, has been prepared⁴ from ethyl iodide and silver sulfite-S³⁵ in ether.

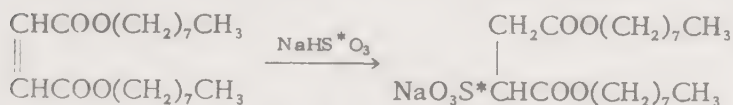
¹A. Schöberl, Hoppe-Syler's Z. physiol. Chem., 216, 193 (1933); Chem. Abstracts, 27, 2937 (1933).

²O. W. Portman and G. V. Mann, J. Biol. Chem., 213, 733 (1955).

³Organic Syntheses, Coll. Vol. II, Wiley, New York, 1943, p. 564.

⁴A. I. Broskiĭ and L. L. Chervyatsova, Doklady Akad. Nauk S.S.S.R., 90, 545 (1953); through Chem. Abstracts, 49, 12272 (1955).

DIOCTYL SODIUM SULFOSUCCINATE-S³⁵



D. J. Salley, A. J. Weith, Jr., A. A. Argyle and J. K. Dixon, Proc. Roy. Soc. (London), A203, 42 (1950).

A. Procedure

Using high-vacuum techniques, sodium carbonate (3.3 mg. as a 10% solution) is deposited by freeze-drying as a thin layer of solid on the walls of a small ampoule. Then, 6.8 mg. of water and 31 mg. of an absolute alcohol solution (Note 1) of octyl maleate (0.062 mmole) are added. After the mixture is frozen in liquid nitrogen and the system is evacuated, the sulfur-S³⁵ dioxide from 12 mg. of barium sulfate-S³⁵ (Note 2) is distilled into the ampoule. The ampoule is then sealed and rotated for about 8 hours in an oil-bath at 80°. Following the heating period, the reaction mixture is evaporated to dryness *in vacuo*. The residue is extracted with 3 portions of carbon tetrachloride which are combined, filtered and evaporated to dryness in a centrifuge cone. To the 18.5 mg. of crude product is added 52.5 mg. of inactive dioctyl sodium sulfosuccinate, as carrier, and the whole is dissolved in the minimum amount of 75% alcohol at room temperature. When the solution is cooled to 1°, the product crystallizes and the mother liquor is removed with a filter stick. The recrystallization is repeated 5 times; the final weight of the product is about 10 mg. (Note 3).

B. Notes

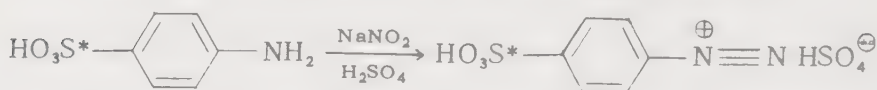
1. Octyl maleate was dissolved in absolute ethanol to give 0.002 mole of ester per gram of solution.

2. Barium sulfate-S³⁵ (12 mg.) was reduced with an equal weight of finely powdered iron at 1200° for one hour in a stream of dry nitrogen.

The sulfur-S³⁵ dioxide, in 98% yield, was collected in a U-tube cooled with liquid air.

3. Between the second and fifth recrystallizations, the specific activity of the product increased only 10%. The yield, based on specific activities of starting material and product, was 10%.

p-SULFOBENZENEDIAZONIUM-S³⁵ BISULFATE



M. Sonenberg, A. S. Keston, W. L. Money and R. W. Rawson, *J. Clin. Endocrinol. and Metab.*, **12**, 1269 (1952).

A. Procedure

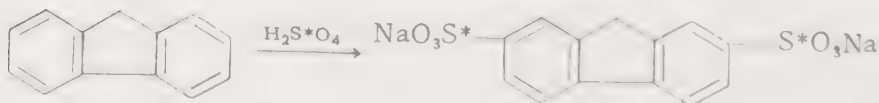
To 50 μc . of sulfanilic-S³⁵ acid, in an ice-bath, is added 10 μg . of ordinary sulfanilic acid (in 0.1 ml. of water), 0.1 ml. of 0.2 M sodium nitrite solution and, dropwise, 0.1 ml. of 0.18 N sulfuric acid. The excess nitrous acid is destroyed with 0.1 ml. of 1 M ammonium sulfamate (Notes 1 and 2).

B. Notes

1. The resulting solution of *p*-sulfobenzenediazonium-S³⁵ bisulfate was added to 3.3 mg. of thyrotropic hormone preparation dissolved in 0.1 ml. of a 1 M solution of sodium carbonate-sodium bicarbonate, pH 8.8. After 1 hour at 4°, this mixture was transferred to a Visking dialysis tube. The preparation was dialyzed at 4°, with mechanical stirring against four 17-1. changes of isotonic saline solution during 2 days. Only traces of isotopic sulfur were found in the third dialysate and none in the fourth. The solution was then adjusted to a concentration of approximately 300 μg . of thyrotropic hormone preparation per ml., for injection purposes.

2. Bovine serum albumin preparations were labeled in a similar fashion with *p*-sulfobenzenediazonium-S³⁵ bisulfate.

SODIUM 2,7-FLUORENEDISULFONATE-S₂³⁵



M. F. Argus, *Brit. J. Cancer*, **7**, 273 (1953).

A. Procedure

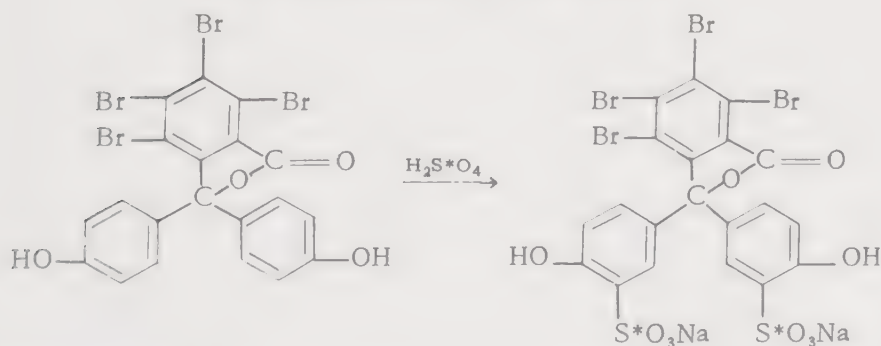
A mixture of 5 g. (0.003 mole) of fluorene and 6.9 ml. (0.12 mole) of sulfuric-S³⁵ acid is warmed on a steam-bath. After about 30 minutes, the fluorene dissolves. The solution is warmed an additional 1½ hours, during which time a white precipitate forms. As cracked ice is added, most of the material dissolves in the aqueous solution, which is filtered using a filter stick. The product is then precipitated by the addition of a saturated solution of sodium chloride. The crude product is dissolved in a minimum of boiling water and filtered while hot. To the clear filtrate is added absolute alcohol to incipient turbidity, and the product soon crystallizes. After this procedure is repeated, the yield of sodium 2,7-fluorenedisulfonate-S₂³⁵ is 10.6 g. (47.71%), based on sulfuric-S³⁵ acid (Notes 1 and 2).

B. Notes

1. The yield based on fluorene was 95.42%.
2. 2,7-Fluorenebis(sulfon-*p*-toluidide)-S₂³⁵ was prepared and melted at 326°. 2,7-Fluorenedisulfonyl-S₂³⁵ chloride was also prepared, m.p. 225-226°, corresponding to that reported by Courtot.¹

¹C. Courtot and R. Geoffroy, *Compt. rend.*, 178, 2259 (1924).

4,4'-(TETRABROMOPHTHALIDYLIDENE)BIS(1-PHENOL-2-SULFONATE)-S₂³⁵
(Sulfobromophthalein-S₂³⁵ Sodium)



J. S. Krebs and R. W. Brauer, USNRDL-TR-67, Nov. 4, (1955).

A. Procedure

A mixture of 24.6 mg. of sulfuric-S³⁵ acid, in dilute aqueous solution, and 15.0 mg. of phenoltetrabromophthalein is heated in a water-bath at 100° and dried, first with an air-stream and finally under vacuum (10⁻² mm.) for about 5 minutes (Note 1). The sulfonation mixture is dissolved in water and neutralized with dilute sodium hydroxide; the solution is

then diluted 7 to 10-fold with acetone. The precipitate of sodium sulfate- S^{35} is removed by centrifugation (Note 2), and the solution is poured onto a column of alumina (Alcoa F-20), 80-200 mesh, 13×75 mm. The sulfonated product is absorbed very readily and appears as a purple band at the top of the column; the unsulfonated dye spreads over the entire column and into the effluent. The column is developed with aqueous acetone (1:1 by volume), containing 400 mg. of sodium sulfate and 4 ml. of concentrated ammonium hydroxide per 100 ml., at a rate not exceeding 12 ml. per hour. The sulfonated dye moves down the column in a relatively narrow band and begins to appear in the effluent after about 25 ml. is collected. The developing solution is passed through the column until all the product is eluted. The eluate is then concentrated by evaporation at room temperature with a stream of nitrogen. The dye is repeatedly crystallized from sodium chloride solution (70-80% saturated) until the specific activity of the product is constant (Note 3).

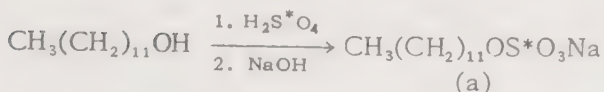
B. Notes

1. The end of the sulfonation reaction is indicated when the mixture becomes a dark-red, viscous liquid. The quantity of acid used corresponds to 5-6 times the amount theoretically required for complete reaction. Reduction of the excess of acid reduced the yield of dye, which in turn defeated an attempted economy of sulfur-35. Prolonged heating under vacuum did not increase the yield of sulfonated product.

2. The amount of sodium sulfate remaining in solution was found in preliminary experiments to be about 0.2-0.3% of the amount originally present. At the same time, the loss of product in the precipitate was 2-3%. The recovered sodium sulfate- S^{35} may be converted to sulfuric- S^{35} acid by dissolution in water and passage of the solution through a narrow glass column containing about 1 gram of Dowex-50 resin in the acid form, pre-washed with water until free of chloride and iron. The column is washed with water until free of S^{35} activity; the effluent is concentrated, and the solution of sulfuric- S^{35} acid may be used in a subsequent sulfonation, without further treatment.

3. The identity of the product with an authentic commercial preparation was established by chromatography and by the constant specific activity of a mixture of the S^{35} -labeled dye with an authentic sample during repeated recrystallization.

SODIUM DODECYL SULFATE-S³⁵
(Sodium Lauryl Sulfate-S³⁵)



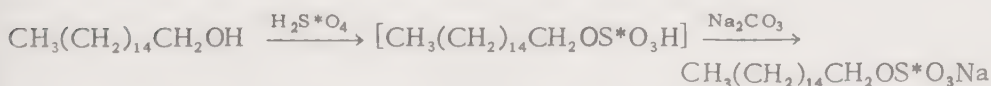
R. Croes and R. Ruysen, Bull. soc. chim. biol., 33, 1837 (1951).

Procedure

(a) *Sodium Dodecyl Sulfate-S³⁵*, (*Sodium Lauryl Sulfate-S³⁵*). A mixture of 300 mg. of lauryl alcohol and 0.25 ml. of concentrated sulfuric-S³⁵ acid is agitated intermittently for 30 minutes. After dilution, the solution is neutralized with sodium carbonate, and the product is precipitated by the addition of a solution of sodium chloride (10%). The product is recrystallized several times from ethyl alcohol.

(b) *Sodium Hexadecyl Sulfate-S³⁵*, (*Sodium Cetyl Sulfate-S³⁵*). In a manner similar to that described above, sodium cetyl sulfate-S³⁵ is prepared from cetyl alcohol and sulfuric-S³⁵ acid at 40°.

SODIUM HEXADECYL SULFATE-S³⁵



G. Aniansson, J. Phys. and Colloid Chem., 55, 1286 (1951).

G. Nilson and O. Lamm, Acta Chem. Scand., 6, 1175 (1952).

A. Procedure

(a) *Sodium Hexadecyl Sulfate-S³⁵*. To a small amount of concentrated sulfuric-S³⁵ acid (Note 1) in a small centrifuge tube is added an excess (Note 2) of pure 1-hexadecanol, with cooling. After mixing the reagents, the resulting alkyl hydrogen sulfate is neutralized with sodium carbonate (Note 3). The sodium alkyl sulfate is then recrystallized from ethanol 5 times before use.

(b) *Sodium Tetradecyl Sulfate-S³⁵*. This compound is prepared, in the manner described above, from anhydrous 1-tetradecanol.

B. Notes

1. The sodium alkyl sulfate was prepared in 50-100 mg. amounts. The carrier-free sulfuric-S³⁵ acid, in 0.1 M hydrochloric acid, was evaporated to dryness and mixed with inactive concentrated sulfuric acid before use.

2. According to Popelier,¹ sulfuric acid may be converted completely to an alkyl hydrogen sulfate by treating it with an excess of anhydrous

alcohol and removing the water formed in the reaction, with some of the unchanged alcohol, by distillation.

3. According to an isolation procedure described by Dreger,² the reaction mixture is mixed with ice, 1-butanol is added, and the solution is neutralized with 2 *N* sodium carbonate solution and sufficient solid sodium bicarbonate to keep the solution saturated with inorganic sodium salts. The neutral sodium alcohol sulfate is separated with the 1-butanol layer, and the aqueous layer is extracted with four successive portions of butanol. By concentrating the combined 1-butanol extracts under vacuum, water is removed, and the precipitated inorganic salts are removed by filtration. Most of the remaining 1-butanol is then removed under vacuum, water is added, and distillation is continued until all the 1-butanol is removed. The aqueous solution is adjusted to pH 7, extracted with ether and then evaporated to dryness.

¹F. Popelier, *Bull. soc. chim. Belg.*, 35, 264 (1926).

²E. E. Dreger, G. I. Keim, G. D. Miles, L. Shellovsky and J. Ross, *Ind. Eng. Chem.*, 36, 610 (1944).

DEXTRAN SULFATE-S³⁵

C. R. Ricketts, *Biochem. J.*, 58, 532 (1954).

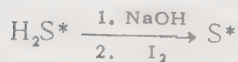
A. Procedure

Chlorosulfonic-S³⁵ acid (0.76 ml.) is added to 7.2 ml. of pyridine, which is cooled in a Dry Ice-ethanol bath, and 0.4 ml. more chlorosulfonic acid is used for washing. When the temperature is raised to 60°, all the pyridinium salts dissolve, 53 g. of dextran is added, and stirring is continued at 70–80° for 4 hours. The solution is cooled and 28 ml. of water is added, followed by sodium hydroxide solution (40% w/v) until pyridine separates. To the aqueous phase is added 30 ml. of ethanol, which precipitates sodium dextran sulfate-S³⁵ as a syrup together with some sodium sulfate. The syrup is dissolved in water and dialyzed (72 hours) until the dialysate gives no precipitate with barium chloride. The solution is neutralized, concentrated under reduced pressure and lyophilized; the yield is 1.09 g. of dextran sulfate-S³⁵ containing 18% sulfur (Note 2).

B. Notes

1. A preparation of dextran, of intrinsic viscosity 0.033, is described by the author.

2. On this basis, 86% of the product was recovered which contained 36% of the available isotope. A trial preparation from 0.53 g. of dextran gave 1.0 g. of sodium dextran sulfate-S³⁵ containing 16.4% sulfur.

SULFUR-S³⁵

J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter and V. du Vigneaud, J. Am. Chem. Soc., 70, 2547 (1948).

A. Procedure

Hydrogen sulfide-S³⁵, from 116 mg. of barium sulfate-S³⁵ *via* the sulfide-S³⁵, is absorbed in 6 ml. of 0.5 *N* sodium hydroxide. In a 50-ml. centrifuge cone are placed 15 ml. of 0.1 *N* iodine in potassium iodide solution and 1 ml. of concentrated hydrochloric acid. The 6 ml. of sodium sulfide-S³⁵ solution is introduced at the bottom of this solution by means of a long slender pipet (Note 1). After 15 minutes, the excess iodine is destroyed with a few drops of freshly prepared solution of stannous chloride in 5 *N* hydrochloric acid. After several hours, the free sulfur-S³⁵ in the resulting suspension coagulates, and is then collected by centrifugation. The precipitate is washed with water by centrifugation and decantation (Note 2).

B. Notes

1. The 1 ml. of sodium hydroxide from a second hydrogen sulfide trap was used to wash the first container and pipet. The two traps were washed further with small portions of water until a nitroprusside or lead acetate test for the sulphydryl group on the washings was negative. The lower portion of the pipet, coated with sulfide solution, was then broken off and placed in the iodine solution.

2. For subsequent use, Wood, *et al.*, dissolved the sulfur-S³⁵ in 10 ml. of hot *m*-xylene. Carbon disulfide has also been used as solvent.²

C. Other Preparations

Sulfur-S³⁵ has been prepared from sulfide-S³⁵, by oxidation with iodine in a number of instances.¹⁻⁵

A study has been made of the formation of free sulfur-S³⁵ from hydrogen sulfide-S³⁵ and sulfur-S³⁵ dioxide in aqueous medium.⁶ The elementary sulfur originates from hydrogen sulfide and sulfur dioxide in the ratio 2:1; therefore, if hydrogen sulfide-S³⁵ is reacted with ordinary sulfur dioxide, in excess, the specific activity of the sulfur-S³⁵ is 66-74% of that of the hydrogen sulfide-S³⁵.

¹A. M. Seligman, A. M. Rutenburg and H. Banks, J. Clin. Invest., 22, 275 (1943).

²R. A. Cooley, D. M. Yost and E. McMillan, J. Am. Chem. Soc., 61, 2970 (1939).

³R. R. Brown, S. Kirkwood, L. Marion, S. Naldrett, R. K. Brown and R. B. Sandin, *ibid.*, 73, 465 (1951).

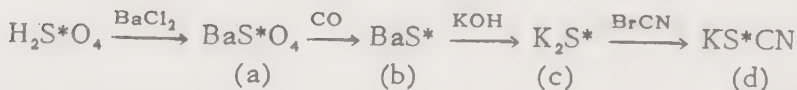
⁴J. L. Wood and L. Van Middlesworth, *J. Biol. Chem.*, 179, 529 (1949).

⁵Y. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, *Doklady Akad. Nauk S.S.S.R.*, 91, 1129 (1953). (1953).

⁶H. B. van der Heijde and A. H. W. Aten, Jr., *J. Am. Chem. Soc.*, 75, 754 (1953).

POTASSIUM THIOCYANATE-S³⁵

METHOD I



L. Eldjarn, *Acta Chem. Scand.*, 7, 343 (1953).

A. Procedure

(a) *Barium Sulfate-S³⁵*. Carrier-free sulfuric-S³⁵ acid (10 mc.) in approximately 0.5 ml. of solution is washed into a solution of 64.2 mg. of carrier sodium sulfate. Barium sulfate-S³⁵ is precipitated, by the addition of barium chloride solution, collected and ignited according to Kolthoff¹ (Note 1). The yield of barium sulfate-S³⁵ is quantitative, 105.5 mg.

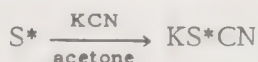
(b) *Barium Sulfide-S³⁵*. The barium sulfate-S³⁵ is transferred to a porcelain boat and carefully spread to give a greater surface area. A quartz reduction tube is preheated to 800–900° and flushed with carbon monoxide (Note 2). The boat containing the barium sulfate-S³⁵ is inserted into the hot tube, and carbon monoxide is passed through the tube at a slow constant rate for 30 minutes. The yield of barium sulfide-S³⁵ is 75.5 mg. (100%) (Note 3), after the boat is cooled to room temperature in a desiccator over phosphorus pentoxide (Note 4).

(c) *Potassium Sulfide-S³⁵*. The apparatus consists of a small cup or portion of a test tube, which stands upright inside a vaccine bottle. The barium sulfide-S³⁵ is transferred into the cup, and into the outer vessel is introduced a solution of 105 mg. of potassium hydroxide in 2 ml. of water. The tight-fitting rubber stopper is replaced, and a new, chromium-plated injection needle is inserted through the stopper. By means of a glass syringe, 1.5 ml. of 10% sulfuric acid is added slowly to the barium sulfide, through the needle. The needle is withdrawn (Note 5), and the vessel is left for 48 hours, with external cooling, while the hydrogen sulfide-S³⁵ is absorbed in the basic solution.

(d) *Potassium Thiocyanate-S³⁵*. The potassium sulfide-S³⁵ solution is left in the diffusion vessel; the cup is washed with 1 ml. of water and

removed. The alkaline potassium sulfide solution is warmed to 80° on a water-bath, and cyanogen bromide (Note 6) is added in three portions of 43 mg., 22 mg. and 22 mg., at 10-minute intervals. Then, 300 mg. of potassium thiocyanate is added as carrier, and the solution is neutralized with 1 *N* hydrochloric acid and evaporated to dryness under reduced pressure. The residue is extracted with 3 portions of boiling ethanol (9 ml., 4.5 ml. and 4.5 ml.) which are combined, filtered and evaporated. The residue is extracted with three 50-ml. portions of ethyl acetate, which are also combined, filtered and evaporated to dryness under reduced pressure. After four crystallizations of the product from absolute ethanol, 266 mg. of potassium thiocyanate- S^{35} , m.p. 172° (uncor.), is recovered.

METHOD II



J. L. Wood, E. F. Williams, Jr., and N. Kingsland, *J. Biol. Chem.*, **170**, 251 (1947).

A. Procedure

Potassium thiocyanate- S^{35} is synthesized according to an adaptation of the method of Castiglioni² (Note 7). In the following example, a mixture of 32 g. of sulfur, 1500 ml. of acetone and 65.1 g. of potassium cyanide is heated under reflux for 45 min. The yield of potassium thiocyanate is 90% of theoretical (Note 8).

B. Notes

1. The techniques of semi-micro organic chemistry might also be applied to the isolation and drying of the barium sulfate precipitate.

2. The carbon monoxide for the reduction is evolved from concentrated formic acid (sp. gr. 1.2, 90%) by the action of concentrated sulfuric acid, as described by Vogel,³ and is passed through a tower filled with potassium hydroxide and a wash-bottle containing concentrated sulfuric acid.

3. In the conversion of barium sulfate to barium sulfide, as described by Kamen,⁴ hydrogen gas is used as the reducing agent at a temperature of $900-1000^{\circ}$. In addition to the explosion hazard, this method has the disadvantage that some of the sulfur is reduced to hydrogen sulfide.

4. Because of the instability of barium sulfide, the next synthetic step is carried out as soon as possible.

5. Because of the slight pressure inside the bottle, the plunger of the syringe is kept depressed while withdrawing the needle.

6. The cyanogen bromide was freshly prepared.⁵

7. The reaction of sulfur with sodium or potassium cyanide takes place in acetone and alcohols at room temperature. The best yield and purest product are obtained with acetone since it dissolves both sulfur and thiocyanates but not the cyanides.

8. In the original literature,² tabulated rate data are also given for the reaction in acetone at 16° and 30°.

C. Other Preparations

Barium sulfate-S³⁵ has been reduced to barium sulfide-S³⁵ with sugar charcoal at 1000°^{6,7} and 900°;⁸ and by passing hydrogen over finely ground barium sulfate-S³⁵ at 750°,⁹ 900°,¹⁰ 1000°,^{8,11,12} and 800°.^{13,14} The latter two references report 98-99% yields. Traces of hydrogen sulfide-S³⁵ formed by a side reaction with hydrogen were collected in suitable absorbing media, e.g., zinc chloride solution.¹³

A solution of potassium thiocyanate-S³⁵ has been prepared,¹⁴ on a micro-scale, from 0.25 g. of sulfur-S³⁵ and 0.05 g. of potassium cyanide.

¹I. M. Kolthoff and E. B. Sandell, *Textbook of Quantitative Inorganic Analysis*, 3rd. ed., The Macmillian Co., New York, 1952, p. 322.

²A. Castiglioni, *Gazz. chim. ital.*, 63, 171 (1933); *Chem. Abstracts*, 27, 3700 (1933).

³A. I. Vogel, *A Textbook of Practical Organic Chemistry*, Longmans, Green and Co., New York, 1951, p. 181.

⁴M. D. Kamen, *Radioactive Tracers in Biology*, Academic Press Inc., New York, 1948, p. 204.

⁵*Organic Syntheses*, Coll. Vol. II, Wiley, New York, 1946, p. 150.

⁶M. J. E. Ernsting and W. Th. Nauta, *Landbouwkundig Tijdschr.*, 61, 903 (1949); through *Chem. Abstracts*, 44, 3444 (1950).

⁷R. A. Peters, G. H. Spray, L. A. Stocken, C. H. Collie, M. A. Grace and G. A. Wheatley, *Biochem. J.*, 41, 370 (1947).

⁸J. C. Boursnell, G. E. Francis and A. Wormall, *Biochem. J.*, 40, 743 (1946).

⁹A. M. Seligman, A. M. Rutenberg and H. Banks, *J. Clin. Invest.*, 22, 275 (1943).

¹⁰R. A. Cooley, D. M. Yost and E. McMillan, *J. Am. Chem. Soc.*, 61, 2970 (1939).

¹¹J. L. Wood, J. R. Rachele, C. M. Stevens, F. H. Carpenter, and V. du Vigneaud, *ibid.*, 70, 2547 (1948).

¹²J. Bell and K. A. MacDonald, *J. Chem. Soc.*, 1951, 1930.

¹³F. C. Henriques, Jr. and C. Margnetti, *Ind. Eng. Chem., Anal. Ed.*, 18, 476 (1946).

¹⁴Y. V. Markova, A. M. Pozharskaya, V. I. Maimind, T. F. Zhukova, N. A. Kosolapova and M. N. Shchukina, *Doklady Akad. Nauk S.S.S.R.*, 91, 1129 (1953).

GENERAL INDEX

The general considerations which served as guides in the formulation of this index are the following:

1. All chemical names are indexed, with the sole exception of common solvents.

2. Page numbers are presented in three modifications: complete syntheses of compounds are indicated in bold-face type, references to preparations without procedural details are given in italics, and the use of compounds as reagents is shown by entries in ordinary type.

3. Index forms of text names are constructed, where feasible, according to the Subject Index usage of Chemical Abstracts.

4. Order is primarily based on nonisotopic parts of names. Secondary considerations, in decreasing order of importance, are element symbol, superscript, subscript and locant.

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 —, 3-[4-(4-hydroxy-3,5-diiodophenoxy)-3,5-diiodophenyl-I¹³¹]-: 1216
 —, 3-[4-(4-hydroxy-3,5-diiodophenoxy-3-I¹³¹)-3,5-diiodophenyl]-: 1212
 —, 3-[4-(4-hydroxy-3,5-diiodophenoxy-I¹³¹)-3,5-diiodophenyl]-: 1212, 1213
 —, 3-[4-(4-hydroxy-3,5-diiodophenoxy-3-I¹³¹)-*m*-iodophenyl]-: 1212
 —, 3-[4-(4-hydroxy-3,5-diiodophenoxy-I¹³¹)-*m*-iodophenyl]-: 1212
 —, 3-(4-hydroxy-3,5-diiodophenyl)-, -1-C¹⁴: 231
 —, 3-[4-(4-hydroxy-3-iodophenoxy-I¹³¹)-3,5-diiodophenyl]-: 1212
 —, 3-[4-(4-hydroxy-3-iodophenoxy-I¹³¹)-*m*-iodophenyl]-: 1211
 —, 3-[4-(*p*-hydroxyphenoxy)-3,5-diiodophenyl]-, -1-C¹⁴; and hydrochloride: 234
 —, 3-[4-(*p*-hydroxyphenoxy)-3,5-diiodophenyl]-, -I¹³¹: 1216
 —, 3-(*p*-hydroxyphenyl)-, -1-C¹⁴: 231, 232
 —, 3-(*p*-hydroxyphenyl)-, -2-C¹⁴: 225
 —, 3-(*p*-hydroxyphenyl)-, -3-C¹⁴: 228
 —, *N*-iodoacetyl-3-phenyl-, -I¹³¹: 1205
 —, 3-(*p*-methoxyphenyl)-, -1-C¹⁴: 232
 —, phenyl-: 1664
 —, phenyl-; L-: 302
 —, 3-phenyl-, -1-C¹⁴: 218
 —, 3-phenyl-, -2-C¹⁴: 226
 —, 3-phenyl-, -3-C¹⁴: 221, 222
 —, 3-phenyl-, -4-C¹⁴; D-: 224; L-: 223
 —, 3-phenyl-, -1,2-C¹⁴: 220
 —, phenyl-, -N¹⁵: 1729, 1730, 1751
 —, phenyl-, -N¹⁵; L-: 1752
 —, 3-phenyl-1,3,5-C¹⁴: 222
 —, 3-phenyl-C¹⁴/₄-, -2-C¹³: 220
 —, H²-phenyl-: 1635
 —, phenyl-H₂⁺: 1663
 —; copper salt: 1664
 β-Alanine-, -2-C¹³-1-C¹⁴: 704
 —, -1-C¹⁴: 168, 704
 —, -2-C¹⁴: 168, 704
 —, -3-C¹⁴: 167, 703
 —, -N¹⁵; and hydrochloride: 1740
 —, 2-bromo-; hydrobromide: 1952
 —, *N*-carbamoyl-, -3-C¹⁴: 704
 —; potassium salt: 703
 Albumin, bovine serum: 1247
 —, I¹³¹-bovine serum: 1246
 —, S³⁵-bovine serum: 2002
 Aldol, acet-, -C¹⁴: 622
 Alizarin, C¹⁴-: 725
 —, H²-: 1654
 Allantoin, C¹⁴-: 773
 —, -2-C¹⁴: 774, 775
 —, -4-C¹⁴: 774
 —, -5-C¹⁴: 774
 —, N₂¹⁵-: 1797, 1799, 1804
 Allantoxaidine-, -2-C¹⁴: 773
 —, -4-C¹⁴: 774
 —, -6-C¹⁴: 774
 Allantoxanic acid, -C¹⁴; potassium salt: 774, 775
 —, -2-C¹⁴; potassium salt: 773, 775
 —, -2-C¹⁴; silver salt: 773
 —, -4-C¹⁴; potassium salt: 774
 —, -6-C¹⁴; potassium salt: 774, 775
 —, N¹⁵-; potassium salt: 1798, 1799
 Allene-, -H₂⁺: 1427, 1428, 1496
 Allethrin, C¹⁴-: 465
 —; *trans*-: 468
 Allethrolone: 465, 466
 —, C¹⁴-: 468
 Allocholan acid, C¹⁴-: 1106
 —, 3β-hydroxy-, -3α,4,5α-H₂⁺: 1538
 —, 3-oxo-, -4,5-H₂⁺; methyl ester: 1538
 Allonic acid, -1-C¹⁴; D-, γ-lactone: 996, 997
 —, 2-C¹⁴; D-, γ-lactone: 997
 3β,20-Allopregnanediol-, -5,6-H₂⁺: 1572
 —, 17α,20-epoxy-, -5,6-H₂⁺; diacetate: 1571
 20-Allopregnanone, 3β,17α-dihydroxy-, 3-acetate: 1572
 —, 21-bromo-3β,17α-dihydroxy-, -5,6-H₂⁺; 3-acetate: 1571
 —, 3β,17α-dihydroxy-, -5,6-H₂⁺: 1571

- 20-Allopregnanone (*Continued*)
 —, 3 β ,17 α -dihydroxy-, -5,6-H $_2^2$; 3-acetate: 1571
 —, 3 β -hydroxy-, -5,6-H $_2^2$; acetate: 1571, 1572
 —, 3 β -hydroxy-, -5,6,7-H $_2^2$; acetate: 1567
 —, 3 β ,17 α ,21-trihydroxy-, -5,6-H $_2^2$: 1572
 —, 3 β ,17 α ,21-trihydroxy-, -5,6-H $_2^2$; 3,21-diacetate: 1572
 17-Allopregnene-3 β ,20-diol, -5,6-H $_2^2$; diacetate: 1571
 Allose, -1-C 14 : D-: 997
 Allothreonine, -1-C 14 : 188, 208
 —; ethyl ester: 208
 —, -4-C 14 : 183
 —, -1,2-C 14 : 187
 —, -N 15 : 183, 1748
 —, N-acetyl-; ethyl ester: 185
 —, N-acetyl-, -4-C 14 ; ethyl ester: 182
 —, N-formyl-O-methyl-: 188
 —, N-formyl-O-methyl-, -1,2-C 14 : 187
 Alroketoheptose, -2-C 14 ; D-: 1015
 —; D-, hexaacetate: 1015
 —, 1-deoxy-1-diazo-, -2-C 14 ; D-, pentaacetate: 1015
 Alloxan, -2-C 14 : 713
 —, -N 15 : 1806, 1807
 Allyl alcohol (also see 2-Propen-1-ol), -1-C 14 ; and *p*-toluenesulfonate: 871
 —, -1-H $_2^2$; acetate: 1321
 Allyl bromide (also see Propene, 3-bromo-): 739, 812, 1322, 1527
 —, -1-C 14 : 871
 —, -1,3-C 14 $_2$: 871
 Allyl chloride (also see Propene, 3-chloro-), -1,3-C 14 $_2$: 871
 Altronic acid, -1-C 14 ; D-, cadmium salt: 1014
 —; D-, calcium salt: 996, 1014
 —; D-, pentaacetate: 1015
 —, -2-C 14 ; D-, cadmium salt: 1016
 —; D-, calcium salt: 997
 Altronyl chloride, -1-C 14 ; D-, pentaacetate: 1015
 Aluminon, C 14 : 364
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 Aluminum chloride, -Cl $_3^{36}$: 1193
 Amyl alcohol (also see 1-Pentanol): 1343
 α -Amyra-10, 12-dien-2-ol, -12-H 2 -27-H $_2^1$; acetate: 1586
 α -Amyrene: 1586
 —, -27-H $_2^1$: 1585
 α -Amyren-2-ol, -12-H 2 -27-H $_2^1$; acetate: 1586
 α -Amyr-11-one, 2-hydroxy-, -12-H 2 -27-H $_2^1$; acetate: 1586
 Ammonia-N 15 : 1713, 1716, 1724, 1726, 1727, 1728, 1729, 1731, 1749, 1759, 1763, 1767, 1772, 1790, 1798, 1814, 1843, 1848, 1857, 1859, 1862
 Ammonium bromide, -N 15 : 1716
 —, 2-acetoxyethyltrimethyl-, -N 15 : 1837
 —, (2-acetoxyethyl-C 14) trimethyl-: 966
 —, diethylmethyl[2-(9-xanthenecarbonyloxy-C 14) ethyl]-: 971
 —, dodecyl-1-C 14 trimethyl-: 542, 970
 —, (2-hydroxyethyl-C 14)triethyl-: 966
 —, tetramethylene-2,3-C 14 -bis[trimethyl-yl-: 813
 —, trimethyl (3-phenoxypropyl-1-C 14)-: 734
 —, trimethyl-3-phenylpropyl-3-H $_2^1$ -: 1457
 —, trimethylpropyl-1-C 14 -: 807
 —, trimethyl[3-(2,6-xylyloxy)propyl-1-C 14]-: 734
 Ammonium chloride, -N 15 : 1733, 1792, 1837, 1856, 1857
 —, (2-acetoxyethyl)trimethyl-C 14 : 967
 —, C 14 (5-carboxy-2-methylpentyl)trimethyl-: 1109
 —, (carboxymethyl)trimethyl-; hydrazide: 1093
 —, (carboxymethyl)trimethyl-, -N 15 : 1834
 —, (carboxymethyl)trimethyl-C 14 -: 968, 969
 —, (2-chloroethyl)trimethyl-C 14 -: 962
 —, ethyltrimethyl-C 14 -: 963
 —, (2-hydroxyethyl)trimethyl-, -N 15 : 1836
 —, (2-hydroxyethyl)trimethyl-C 14 -: 961, 962, 969
 —; chloroplatinate: 961
 —, (2-hydroxyethyl)trimethyl-H $_2^2$ -: 1524
 —, (2-hydroxyethyl-1-C 14)trimethyl-: 965
 —; chloroplatinate: 965
 —; mercuric chloride complex: 965
 —, (2-hydroxyethyl-2-C 14)trimethyl-: 963
 —; chloroplatinate: 963
 —; mercuric chloride complex: 963
 —, (3-hydroxypropyl)trimethyl-C 14 -: 964
 Ammonium hydroxide, ethyl-1-C 13 -trimethyl-: 201, 915
 —, ethyl-C 13 -trimethyl-: 915
 —, (2-hydroxyethyl)trimethyl-, -N 15 : 1836
 —, (2-hydroxyethyl)trimethyl-C 14 -: 962

- , tetramethylene-2,3- C_{14}^1 -bis trimethyl-yl-: 814
- , trimethylpropyl-1- C_{14}^1 -: 807
- , trimethyl(3-*p*-tolylloxypropyl-1- C_{14}^1)-: 732
- Ammonium iodide, [2-(diphenylmethoxy- C_{14}^1) ethyl] trimethyl-: 525
- , ethyltrimethyl- C_{14}^1 -: 963
- , ethyl-1- C_{13}^1 -trimethyl-: 915
- , ethyl- C_{13}^1 -trimethyl-: 915
- , hexamethylenebis(trimethyl-, - C_{14}^1): 970
- , (2-hydroxyethyl) trimethyl- C_{14}^1 -: 961
- , (2-hydroxyethyl-1- C_{14}^1)trimethyl-: 965
- , tetramethyl-: 1838
- , tetramethyl-, - C_{14}^1 : 863
- , tetramethyl-, - H_2^1 : 1710
- , H^2 -tetramethyl-: 1651
- , trimethyl(3-phenoxypropyl-1- C_{14}^1)-: 734
- , trimethyl(3-*p*-tolylloxypropyl-1- C_{14}^1)-: 732
- , trimethyl [3-(2,6-xylyloxy)propyl-1- C_{14}^1]-: 734
- Ammonium nitrate, - N^{15} : 1846, 1861
- Ammonium nitrate- N^{15} , tetramethyl-: 1838
- Ammonium reineckate; 1524
- Ammonium sulfate, - N^{15} : 1857
- , 3-indolylmethyl- C_{14}^1 trimethyl-: 337
- Amphetamine, C_{14}^1 ; sulfate: 513
- Amyl alcohol; acetate: 1890
- 3,5-Androstadien-17 β -ol, 3-ethoxy-17 α -ethynyl- C_{14}^1 -: 1807
- 2,4-Androstadien-17-one, 3-ethoxy-: 1084, 1086
- , H^2 -3-ethoxy-: 1560
- 3,5-Androstadien-17-one, 3-ethoxy-: 1087, 1088
- , H^2 -3-ethoxy-: 1560
- 3 α -Androstanol; acetate- H_3^1 : 1545
- 3 β -Androstanol; acetate- H_3^1 : 1545
- 17 β -Androstanol; acetate- H_3^1 : 1545
- 3-Androstanone: 1555
- , -2,4- H_2^1 : 1555
- 17-Androstanone, H_3^1 -: 1555
- , C_{14}^1 -3 β -hydroxy-: 1105
- 1,4,6-Androstatriene-3,17-dione: 1563
- 5-Androstene-3 β , 17 β -diol; diacetate: 1103
- , -7- H_2^1 ; and 3-acetate, 17-benzoate: 1543
- , -7- H_2^1 : 1544
- , —; 3-acetate, 17-benzoate: 1543
- , 7-bromo-; 3-acetate, 17-benzoate: 1543
- , 17 α -methyl- C_{14}^1 -: 1085
- ; acetate: 1086
- 4-Androstene-3, 17-dione: 1560, 1696
- , C_{14}^1 -; 1076, 1082
- ; 17-monosemicarbazone: 1082
- , H^2 -: 1535, 1559, 1560, 1562
- ; 3-enol ether (also see 3,5-androstadien-17-one, H^2 -3-ethoxy-): 1560
- , H^3 -: 1696
- 4-Androsten-3-one, 17 β -hydroxy-, -11, 12- H_2^1 ; acetate: 1558
- 5-Androsten-7-one, 3 β , 17 β -dihydroxy-; diacetate: 1103
- 4-Androstene-3, 11, 17-trione, -4, 16- H_3^1 : 1699
- 5-Androsten-17-one, 3 β -hydroxy-: 1085, 1561
- ; acetate: 1556, 1561
- , 3 β -hydroxy-, -16- C_{13}^1 ; acetate: 1096
- ; semicarbazone: 1096
- , C_{14}^1 -3 β -hydroxy-; semicarbazone: 1082
- ; acetate, semicarbazone: 1081
- , H^2 -3 β -hydroxy-; and acetate: 1535, 1561
- Anethole: 1528, 1692
- β -Angelica lactone, O_1^{18} -: 1874
- Aniline: 397, 545, 1229, 1231, 1248, 1309, 1829, 1854, 1944, 1946, 1962
- ; hydrochloride: 1441
- ; hydrogen phthalate: 976
- ; hydrogen sulfate: 1946
- , -1- C_{14}^1 ; hydrochloride: 105, 322, 600, 892
- , -1, 2- C_{14}^1 : 107, 539
- , - C_{14}^1 : 420
- ; hydrochloride: 323
- , H^2 -; hydrochloride: 1626
- , -2- H^2 : 1376
- , -3- H^2 ; and hydrochloride: 1376
- , - N - H_2^1 : 1371, 1626
- , -2,4,6- H_3^1 : 1389, 1441
- , - N , N , 2,4,6- H_3^1 : 1441
- , -2,3,4,5,6- H_3^1 : 1307, 1309
- , - N^{15} : 1723, 1726, 1727, 1831, 1833, 1839, 1854
- ; hydrochloride: 1727
- , *o*-(2-amino-2-methylvinyl- N^{15})-: 1832
- , *o*-(2-aminopropenyl- N^{15})-: 1832
- , *m*-bromo-: 1376
- , *m*-bromo-, - Br^{82} : 1151
- ; hydrochloride: 1152
- ; phosphate: 1152
- ; picrate: 1152
- , *o*-bromo-: 1376

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- , *o*-bromo-, -Br⁸²: 1151
- ; hydrochloride: 1152
- ; picrate: 1152
- , *p*-bromo-: 318, 1376
- , *p*-bromo-, -Br⁸²: 1151, 1154
- ; hydrochloride: 1152
- ; phosphate: 1152
- ; picrate: 1152
- , H²-*m*-bromo-*N,N*-dimethyl-: 1628
- , *p*-chloro-: 1232
- , *p*-chloro-, -1-C¹⁴; hydrochloride: 600
- , *p*-chloro-, -Cl³⁶; hydrochloride: 1180
- , *p*-chloro-, -N¹⁵: 1854
- , *N,N*-dimethyl-: 543, 1727
- , *N,N*-dimethyl-, -N¹⁵: 1723
- , H²-*N,N*-dimethyl-: 1629
- , *N,N*-dimethyl-C¹³-: 540
- ; hydrochloride: 541
- , *N,N*-dimethyl-C¹⁴-: 541
- , *p*-dimethylamino-, -*N*-N¹⁵: 1725
- , H²-*N,N*-dimethyl-*m*-nitro-: 1628
- , H²-*N,N*-dimethyl-*o*-nitro-: 1629
- , H²-*N,N*-dimethyl-*p*-nitro-: 1629
- , *N,N*-dimethyl-*p*-phenylazo-, -N¹⁵: 1723
- , *N,N*-dimethyl-*p*-phenylazo-1-C¹⁴-: 542
- , *N,N*-dimethyl-*p*-phenylazo-1-N¹⁵-: 1725
- , *N,N*-dimethyl-*p*-phenylazo-2-N¹⁵-: 1727
- , *N,N*-dimethyl-C¹³-*p*-phenylazo-: 540
- , *N,N*-dimethyl-C¹⁴-*p*-phenylazo-: 541
- , *N,N*-dimethyl-C¹⁴-*p*-phenylazo-: 542
- , *N,N*-dimethyl-*p*-(*m*-tolylazo- α -C¹⁴)-: 543
- , *N,N*-dimethyl-C¹⁴-*p*-(*m*-tolylazo)-: 541
- , *N,N*-dimethyl-C¹⁴-*p*-(*p*-tolylazo)-: 542
- , *p*-iodo-, -I¹³¹: 1221, 1248
- ; hydrochloride: 1221
- , 4-methoxy-4-nitro-: 368
- , 4-methoxy-2-nitro-, -N-N¹⁵: 1814
- , 4-methoxy-2-nitro-, -2-N¹⁵: 1818
- , *N*-methyl-: 540, 541
- ; hydrochloride: 608
- , *N*-methyl-*p*-phenylazo-: 541
- , *N*-methyl-C¹⁴-*p*-phenylazo-: 542
- , *N*-methyl-C¹⁴-*p*-(*p*-tolylazo)-: 542
- , *m*-nitro-: 1375
- , *p*-nitro-: 319, 1592, 1721, 1823, 1825
- , *p*-nitro-, -N-N¹⁵: 1724
- , *p*-phenylazo-: 542
- , *p*-(*p*-tolylazo)-: 542
- , 2,4,6-tribromo-, -1,2-C¹⁴₂: 107
- , 2,4,6-tribromo-, -3,5-H²₂: 1310
- , H²-2,4,6-tribromo-: 1377
- m*-Anisaldehyde, 4- β -D-glucosyloxy-, -C¹⁴: 1038
- , 4- β -L-glucosyloxy-, -C¹⁴: 1038
- , 4-(tetra-*O*-acetyl- β -D-glucosyloxy)-, -C¹⁴: 1037, 1038
- , 4-(tetra-*O*-acetyl- β -L-glucosyloxy)-, -C¹⁴: 1038
- p*-Anisaldehyde: 151, 671
- , -C¹⁴: 227
- ; 2,4-dinitrophenylhydrazone: 228
- p*-Anisamide, -C¹⁴: 150
- , *N,N'*-ethylenebis(3-chloro-, -Cl³⁶): 1182
- p*-Anisanilide, 3-chloro-, -Cl³⁶: 1182
- m*-Anisic acid, -O¹⁸: 1871
- , 4-benzyloxy-, -C¹⁴: 98, 229, 325
- , 2-nitro-, -C¹⁴: 321
- o*-Anisic acid, -C¹⁴: 97, 736
- , 3,5-dicarboxy-, -C¹⁴; and triethyl ester: 741
- p*-Anisic acid: 1182
- , -C¹⁴: 92, 97, 150, 227, 325, 743
- , -O¹⁸: 1871
- , 3-chloro-, -Cl³⁶: 1182
- ; ethylene diester: 1182
- ; methyl ester: 1182
- ; phenyl ester: 1182
- ; *o*-phenylene diester: 1182
- o*-Anisidine, H²-: 1627
- p*-Anisidine: 150
- Anisole: 667
- , H²-: 1645, 1653
- , -2-H²: 1380, 1506
- , -3-H²: 1506
- , -4-H²: 1380, 1506
- , 2-allyl-1-C¹⁴-6-allyl-: 740
- , 2-allyl-1-C¹⁴₁-4-allyl-3-C¹⁴₁-6-allyl-: 740
- , 4-allyl-3-C¹⁴-2,6-dimethyl-: 735
- , 4-allyl-1,3-C¹⁴₂-2,6-dimethyl-: 738
- , 2-allyl-1-C¹⁴-4-methyl-: 623
- , 2-benzyloxy-5-bromo-: 94
- , *o*-bromo-: 97, 1380
- , *p*-bromo-: 97, 1380
- , *p*-(3-bromopropyl)-: 97
- , *p*-(α -chlorobenzyl)-: 741
- ; *p*-(1-chloropropyl-2-H²₁)-: 1528
- , *p*-(1-chloropropyl-2-H³₁)-: 1692
- , H²-3,5-dimethyl-: 1645
- , 3-iodo-2-nitro-: 320
- , H²-*m*-methyl-: 1645
- , H²-*o*-methyl-: 1645
- , *p*-propyl-: 1529

- m*-Anisonitrile, 2-nitro-, -C¹⁴: 320
o-Anisonitrile, 4-nitro-, -C¹⁴: 368
m-Anisoyl chloride, 4-benzyloxy-, -C¹⁴: 229
p-Anisoyl chloride, -C¹⁴: 227
 —, 3-chloro-, -Cl³⁶: 1182
 —, 2-nitro-: 1814, 1815
 Anisyl alcohol, α -ethyl-: 1528
 Anthracene, -9-C¹⁴: 849
 —, H²-: 1639
 —, -9-H²: 1467
 —, -9, 10-H₂²: 1466
 —, 9-bromo-: 82
 —, 9-bromo-, -Br⁸²: 1177
 —, 9,10-dibromo-, -Br⁸²: 1177
 —, 9,10-dihydro-9,10-dimethoxy-9,10-diphenyl-: 616
 —, 2,6-dimethyl-, -8,9-H_{1/2}²: 1532
 —, 2-methyl-, -8,9-H_{1/2}²: 1531
 —, 9-methyl-: 84
 9-Anthraceneacetamide, *N,N*-diethyl-9,10-dihydro-, -C¹⁴: 83
 9-Anthraceneacetic acid, 9,10-hydro-, -C¹⁴: 83
 9-Anthracenecarbonitrile, -C¹⁴: 82
 9-Anthramide, -C¹⁴: 83
 Anthranilaldehyde: 785
 Anthranilic acid; copper salt: 1766
 —, -C¹⁴: 316
 —, H²-: 1597
 —, -N¹⁵: 1727, 1766, 1809
 —; copper salt: 1810
 —, *N*-acetyl-, -C¹⁴: 316
 —, *N*-carboxymethyl-, -N¹⁵: 1809
 —, 3-hydroxy-, -C¹⁴: 320
 Anthraquinone, -9-C¹⁴: 848
 —, 1,4-bis(hydroxy-H²)-: 1533
 —, 1,5-bis(hydroxy-H²)-: 1533
 —, 1,2-dihydroxy-, -9-C¹⁴: 725
 —, 2,6-dimethyl-, -8-H²: 1533
 —, 2-hydroxy-, -9-C¹⁴: 725
 —, 2-methyl-, -8-H²: 1531
 —, 1,4,5,8-tetrakis(hydroxy-H²)-: 1533
 Anthrarufin, H₂²: 1533
 9-Anthroic acid, 9,10-dihydro-, -C¹⁴: 83
 Anthrone: 1466
 —, -9,10-C_{1/2}¹⁴: 848
 —, 2-hydroxy-, -9-C¹⁴: 725
 9-Anthronitrile, -C¹⁴: 82
 9-Anthroyl chloride, 9,10-dihydro-, -C¹⁴: 83
 Apresoline, C₁¹⁴-: 792
 Arabinose; D-: 991, 993, 1006
 —; L-: 996
 —; D-, phenyllosazone: 977
 —, O₁¹⁸-: 1895
 —, -1-C¹⁴; D-: 974, 978, 995, 996
 —; L-: 976
 —, -5-C¹⁴; D-: 979
 —, 3-O- β -D-galactopyranosyl-; D-: 1018, 1019
 —, 3-O- α -D-glucosyl; D-: 1022
 Arabitrol, 1-deoxy-1-nitro-, -1-C¹⁴; sodium salt: 975
 Arabonic acid, 1-C¹⁴; D-: 974, 978
 —; D-, calcium salt: 977, 978
 —; D-, γ -lactone: 974
 —; D-, potassium salt: 973
 —, -5-C¹⁴; D-: 979
 —; D-, γ -lactone: 979
 —; D-, potassium salt: 979
 Arabononitrile; L-, tetraacetate: 1862
 —, -1-C¹⁴; D-: 974
 Arginine; L-; copper chloride complex: 273
 —, C₁¹⁴-; L-; monohydrochloride: 272
 —; L-, monoflavianate: 273
 —, N_{1/2}¹⁵-, monoflavianate: 1755
 —; L(+)-; monoflavianate: 1755
 —; L(+)-, monohydrochloride: 1755
 —, N²-*p*-toluenesulfonyl[N_{1/2}¹⁵-: 1755
 —; L(+)-: 1755
 Arsonium iodide, H²-tetramethyl-: 1651
 Arterenol, C₁¹⁴-: 949
 Ascorbic acid, -1-C¹⁴; L-: 1124, 1125
 —, -C₈¹⁴; L-: 1127
 —; L-, lead salt: 1128
 —, dehydro-, -1-C¹⁴: 1125
 —, imino-, -1-C¹⁴; L-: 1124
 —, 2,3-O-isopropylidene-, -C¹⁴; L-: 1124
 Aspartic acid: 253, 305, 1714
 —; L-: 253
 —; hydrochloride: 253
 —, -3-C¹³-4-C¹⁴: 253, 702
 —, -3-C¹⁴: 253, 451
 —; hydrochloride: 253
 —, -4-C¹⁴: 253, 254, 305, 362
 —; hydrochloride: 255
 —, -2,3-C_{1/2}¹⁴; L-: 256
 —; L-, copper derivative: 256
 —, -2,3-H₂³-N¹⁵: 1794
 —, -N¹⁵: 1729
 —; L-: 1792
 —; L-, copper salt: 1793
 —, *N*-benzoyl-: 253
 —, *N*-carbamoyl-, -C¹⁴: 305
 —, *N*-carbamoyl-, -4-C¹⁴: 255, 359, 362
 —, *N*-carbamoyl-C¹⁴-: 305, 357
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8-Azaadenine, -4,6- $C_{1\frac{1}{2}}^{14}$: 776

8-Azaganine, -2- C^{14} : 777

—, -4- C^{14} : 776, 777

8-Azaxanthine, -4- C^{14} : 777

Azelaic acid: 65

5H-Azepotetrazole, 6,7,8,9-tetrahydro-, -5, 9a- $C_{1\frac{1}{2}}^{14}$: 798

Aziridine, - C_2^{14} : 408, 1709

—, -2,3- H_2^2 : 1707

—, 1,1',1''-phosphinothioylidynetris-, - C_6^{14} : 408

—, 1,1',1''-phosphinothioylidynetris-, - H_{12}^2 : 1708

Azobenzene, - N_1^{15} : 1831, 1833

—, - N_2^{15} : 1833

—, 4-dimethylamino- C_1^{14} : 540

—, 2-ethoxy-2'-methyl- C^{14} : 545

—, 3'-methyl-4-dimethylamino- C_1^{14} : 541

—, 3-methyl- C^{14} -4-dimethylamino-: 543

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Barbituric acid, -2- C^{14} : 713, 1120, 1121

—, H^2 : 1656

—, 5-acetamido-, -1,3- N_2^{15} : 1852

—, 5-amino-, -2- C^{14} : 771

—, 5-amino-, -1,3- N_2^{15} : 1853

—, 5-benzylidene-, -2- C^{14} : 713

—, 5-(butylthio)methyl-5-ethyl-2-thio-, - S^{35} : 1975

—, 5,5-dibromo-: 359

—, 5,5-diethyl-, -2- C^{14} : 712

—, 5-ethyl-5-isopentyl-, -1- N^{15} ; sodium derivative: 1806

—, 5-ethyl-5-(1-methylbutyl)-, -2- C^{14} : 711, 712

—; sodium derivative: 711

—, 5-ethyl-5-(1-methylbutyl)-2-thio-, -2- C^{14} : 712

—, 5-ethyl-5-(1-methylbutyl)-2-thio-, - S^{35} : 1976

—, 5-ureido-, -2- C^{14} : 771

—, 5-ureido- C^{14} : 771

—, 5-ureido-3- N^{15} ; and ammonium derivative: 1851

—, 5-ureido-, -1,3- N^{15} ; potassium derivative: 1853

Benadryl, C_1^{14} : 524

—; methiodide: 525

Benz[a]acridine, 10,12-dimethyl-, -12- C^{14} : 786

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—, 7-methyl-, -7- C^{14} : 785

Benzaldehyde: 78, 146, 220, 300, 545, 669, 671, 713, 788, 993

—; phenylhydrazine: 1221

—, - C^{14} : 78, 151, 154, 222, 348, 530, 626, 627, 628, 629, 897

—; 2,4-dinitrophenylhydrazine: 629

—; sodium bisulfite addition compound: 789

—, - C^{14} -1,2,3,4- $C_{1\frac{1}{4}}^{14}$; and semicarbazone: 108

—, -1,2,3,4- $C_{1\frac{1}{4}}^{14}$: 219

—; sodium bisulfite addition compound: 220

—, - $C_{1\frac{1}{2}}^{14}$: 109

—, H^2 : 1605

—, - H^2 : 1365, 1366, 1367

—; sodium bisulfite addition compound: 1365, 1366

—, - O^{18} : 1896

—, o-amino-: 785

—, o-bromo-; diethyl acetal: 791, 792

—, p-chloro-, - H^2 : 1367

—, 3,5-diiodo-4-(p-methoxyphenoxy)-: 233

—, 3,4-dimethoxy-, - C^{14} : 229

—, o-hydroxy-: 1368

—, o-hydroxy- H^2 : 1367

—, p-hydroxy-: 1762

—, 4-hydroxy-3,5-dimethoxy-, - C^{14} : 229

—, 4-hydroxy-3-methoxy-, - C^{14} : 229, 1037, 1038

—, p-isopropyl-; thiosemicarbazone- S^{35} : 1976

—, p-phenyl-, -1- H^2 ; and sodium bisulfite addition compound: 1366

—, p-(tetra-O-acetyl-β-D-glucosyloxy)-: 1035

Benzamide: 1727

—, - C^{14} : 394, 627, 678

—, -3,4,5- H_3^2 : 1280

—, - N^{15} : 1726, 1727

—, - O^{18} : 1886

—, N-(2-bromoethyl)-: 2000

—, N-(p-bromophenyl)-, - Br^{82} : 1154

—, N,N'-(C^{14} -1,3-cyclopentylene) bis-; cis: 425, 430

—, N-(cyclopropylmethyl- C^{14})-: 506

—, N,N'-(dithiodiethylene)di-, - S_2^{35} : 2000

—, N-2-fluorenyl-9- C^{14} : 386

—, N-2-mercaptoethyl-, - S^{35} : 2000

—, 4-methoxy-2-nitro-, - N^{15} : 1814

—, N-methyl- H_3^2 : 1314, 1470, 1504

—, N-(α-methylcinnamyl-α- C^{13})-: 301

—, N-(α-methylphenethyl-β- C^{14})-: 513

—, p-nitro-, -N- N^{15} : 1724

—, C^{14} -N,N'-octamethylenebis-: 538

—, N-phenyl-, - C^{14} : 922

- , *N*-phenyl-, -N¹⁵: 1727
 —, thio-, -S³⁵: 1977
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 —; picrate: 852
 —, 7,12-dimethyl-, -7,12-C¹⁴_{1/2}: 849
 —, 7-iodomethyl-12-methyl-, -7,12-C¹⁴_{1/2}: 850
 Benz[*a*]anthracene-5,6-diol,5,6-dihydro-, -5,6-C¹⁴_{1/2}: 685
 Benz[*a*]anthracene-5,6-dione, -5,6-C¹⁴_{1/2}: 685
 Benz[*a*]anthracene-7,12-dione, -7,12-C¹⁴_{1/2}: 849
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 —; clathrate: 822
 —, H²—: 1637
 —, -H¹₁: 1376, 1437, 1438, 1506, 1509
 —, -1,2-H¹₂: 1440
 —, -1,3-H¹₂: 1440
 —, -1,4-H¹₂: 1439, 1440
 —, -1,3,5-H¹₃: 1441, 1442
 —, -1,2,4,5-H¹₄: 1444
 —, -1,2,4,6-H¹₄: 1443
 —, -H¹₂: 1445, 1446
 —, -H²₂: 1274, 1280, 1307, 1372, 1393, 1397, 1445, 1446, 1447, 1466, 1467, 1508
 —, -H¹₃: 1674, 1684, 1688, 1689, 1705
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 —, allyl-1-H¹₁—: 1457
 —, azido-, -1-N¹⁵: 1854, 1855, 1856
 —, azido-, -2-N¹⁵: 1854, 1855
 —, azido-, -2,3-N¹⁵₂: 1855
 —, *m*-bis(ethyl-2-C¹⁴)—: 835
 —, *p*-bis(ethyl-2-C¹⁴)—: 835
 —, bromo—: 88, 109, 840, 886, 922, 1684
 —, bromo-, -Br⁸⁰: 1158, 1176
 —, bromo-, -Br⁸²: 1157, 1158, 1160, 1176
 —, bromo-, -C¹⁴_{1/4}: 841, 883, 926
 —, 1-bromo-, -4-H²: 1439
 —, bromo-, -H¹₂: 1281, 1374, 1462, 1444, 1445
 —, 1-bromo-, -2-H³: 1675, 1684
 —, 1-bromo-, -3-H³: 1675, 1684
 —, 1-bromo-, -4-H³: 1675, 1684
 —, 1-bromo-4-chloro—: 97
 —, 1-bromo-2,4-dinitro-, -Br⁸²: 1176
 —, (1-bromoethyl)-, -Br⁸²: 1174
 —, (2-bromoethyl)-: 1487
 —, (2-bromoethyl-C¹⁴_{1/2})—: 884
 —, 1-bromo-2-fluoro—: 1506
 —, (3-bromo-2-methylpropyl)—: 1143
 —, 1-bromo-2-nitro-, -Br⁸²: 1151, 1160, 1176
 —, 1-bromo-3-nitro-, -Br⁸²: 1151, 1160
 —, 1-bromo-4-nitro—: 43
 —, 1-bromo-4-nitro-, -Br⁸²: 1151, 1161
 —, (3-bromopropyl)—: 92
 —, (3-bromopropyl-1-H¹₃)—: 1457
 —, chloro—: 665, 888, 889
 —, chloro-, -Cl³⁶: 1187
 —, 1-chloro-, -1-C¹⁴: 539, 892
 —, 1-chloro-, -C¹⁴_{1/4}: 892
 —, (1-chloroethyl)-; D- and L-: 1591
 —; (—): 1590
 —, (2-chloroethyl-2-C¹⁴)—: 76, 884
 —, (2-chloroethyl-C¹⁴_{1/2})—: 884
 —, 1-chloro-4-iodo—: 97
 —, 1-chloro-4-iodo-, -I¹³¹: 1232
 —, (2-chloro-2-methylpropyl)—: 1451
 —, 1-chloro-2-nitro—: 1387
 —, 1-chloro-2-nitro-, -Cl³⁶: 1189
 —, 1-chloro-3-nitro-, -Cl³⁶: 1188
 —, 1-chloro-4-nitro—: 1387
 —, 1-chloro-4-nitro-, -Cl³⁶: 1188
 —, *m*-dibromo—: 1684
 —, *o*-dibromo—: 1684
 —, *p*-dibromo—: 1443, 1684
 —, *p*-dibromo-, -H¹₂: 1443, 1445
 —, *p*-dibromo-, -2-H³: 1674, 1675
 —, H³-*p*-dibromo—: 1675
 —, (1,2-dibromoethyl-1-C¹⁴)—: 837
 —, (1,2-dibromoethyl-2-C¹⁴)—: 837
 —, H²-1,2-diisobutoxy—: 1645
 —, *m*-dinitro-, -1,2,4,5-C¹⁴₄: 895
 —, *m*-dinitro-, -2,4,5-H¹₃: 1509
 —, *m*-dinitro-, -2,4,5-H¹₃: 1689
 —, *m*-dinitro-, -N¹⁵₂: 1833
 —, *o*-dinitro-, -1,3,4-C¹⁴₃: 895
 —, *p*-dinitro-, -1,2-C¹⁴_{1/2}: 895
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 —, ethyl-2-C¹⁴—: 834
 —, ethyl-C¹⁴_{1/2}—: 836
 —, ethyl-1-H¹₁—: 1484, 1590
 —, ethyl-1,1,2,2-H²₄—: 1455
 —, 1-fluoro-, -1-C¹⁴: 105
 —, 1-fluoro-, -2-H²: 1505
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 —, H²-hexamethyl—: 1639
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 —, 1-iodo-3-nitro-, -I¹³¹: 1233, 1257
 —, 1-iodo-4-nitro-, -I¹³¹: 1233, 1257
 —, iodoso-, -I¹³¹: 1245
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 —, (2-methylpropyl-2- H^2)-: 1451
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 —, nitro-, - C_1^{14} : 894
 —, 1-nitro-, -3- H^2 : 1375, 1376
 —, 1-nitro-, -4- H^2 : 1592
 —, nitro-, - H_2^2 : 1307, 1507
 —, 1-nitro-, -2,3,4- $H_{1/3}^2$: 1508
 —, nitro-, - N^{15} : 1727, 1834
 —, nitroso-: 1831
 —, H^2 -pentamethyl-: 1639
 —, propyl-2- C^{14} : 836
 —, 1,3,5-tribromo-: 1442
 —, 2,4,5-tribromo-1-iodo-: 1444
 —, H^2 -1,3,5-trinitro-: 1650
 —, vinyl-: 1455
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 —, - C_1^{14} ; and diethyl ester: 458
 —, - α , α' - C_2^{14} ; diethyl ester: 459
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 —, - C_1^{14} : 458
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 —, - α - N^{15} : 1854, 1855, 1856, 1863
 —, - β - N^{15} : 1829, 1855
 —, *p*-iodo-, - I^{131} : 1221
 —, *p*-nitro-: 896
 —, *p*-nitro-, - β - N^{15} : 1721, 1823
 —; 2-naphthol-1-sulfonate: 1823
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 —, *p*-amino-, - S^{35} : 1947
 —, 4-amino-3-iodo-, - I^{131} : 1241
 —, *N*-ethyl-1- C^{13} -*p*-bromo-: 203
 —, *N*-ethyl-1- C^{14} -*p*-bromo-: 501
 —, *N,N'*-pentamethylenebis-: 540
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 —, *p*-acetamido-, - S^{35} : 1947
 —; sodium salt: 1948
 —, *m*-amino-, - S^{35} : 1946
 —, *o*-amino-, - S^{35} : 1946
 —, *p*-amino-, - S^{35} : 1944, 1946
 —, *p*-diazo-: 1240
 —, *p*-iodo-, - I^{131} ; sodium salt: 1240
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 —, *p*-bromo-: 85, 427, 1329
 —, *p*-iodo-, - I^{131} : 1240
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 —, α -benzyl- α - $C_1^{14}/_1$ -, - α - $C_1^{14}/_1$: 922
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 —, H^2 -: 1630, 1671
 —; dihydrochloride: 1671
 —; monohydrochloride: 1671
 —, - N^{15} ; and sulfate: 1723
 —, *N,N'*-dibenzylidene-3-ethoxy-3'-methyl- C^{14} -: 545
 —, *N,N'*-dibenzylidene-3-methyl- C^{14} -: 545
 —, 5,5'-dibromo-3,3'-dimethyl-, - Br_2^{82} : 1163
 —, 3,3'-dimethyl-: 1163
 —, *N,N'*-di-*p*-toluenesulfonyl-, - S_2^{35} : 1951
 —, 3-ethoxy-3'-methyl- C^{14} -; dihydrochloride: 545
 —, 3-methyl- C^{14} -; dihydrochloride: 545
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 —, C_1^{14} -: 150
 —, - O_2^{18} : 1884
 —, 3,4,3',4'-bis(methylenedioxy)-: 1367
 —, C_1^{14} -4-chloro-: 678
 —, 4,4'-dichloro-: 1367
 —, C_1^{14} -4-methoxy-: 149, 150
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 —, 4-methoxy-, - α - C^{14} : 151
 —, 4-methoxy-, - $C_1^{14}/_1$ - α - $C_1^{14}/_1$: 150
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 —, 5,6-dimethyl-, -2- C^{14} : 789
 —, 2-(1-hydroxyethyl- $C_1^{14}/_2$)-: 927
 —, 2-methyl- $C_1^{14}/_1$ -, -2- $C_1^{14}/_1$; and silver salt: 788
 —, 2-phenyl-, -2- C^{14} ; and hydrochloride: 789
 —, 2-(styryl- β - $C_1^{14}/_1$)-, -2- $C_1^{14}/_1$: 788
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 —, - $C_1^{14}/_1$ -2- $C_1^{14}/_1$: 788
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 —; methyl ester, hydrochloride: 1877
 1*H*-Benz[*e*]indene-6-propionic acid, 3-acetoxy-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl; δ -lactone: 1079
 —, 3-(1-acetoxyethylidene)-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl; δ -lactone: 1066, 1068, 1071

- , 3-acetyldodecahydro-3a,6-dimethyl-7-oxo-: 1071
- , 3-benzoyloxy-2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-; δ -lactone: 1077, 1078
- , 3-carboxydecahydro-3a,6-dimethyl-7-oxo-; dimethyl ester: 1061
- , 2,3,3a,4,5,5a,6,9,9a,9b-decahydro-7-hydroxy-3a,6-dimethyl-3-(1,5-dimethylhexyl)-; δ -lactone: 1097, 1098, 1100, 1101
- 1H-Benz[e]inden-7(2H)-one, 3-(1,5-dimethylhexyl)decahydro-3a,6-dimethyl-6-(3-oxobutyl-4-C¹⁴)-: 1100
- Benz[g]indolo[2,3-a]quinolizine-1-carboxylic acid, 1,2,3,4,4a,5,7,8,13,13b,14,14a-dodecahydro-2,11-dimethoxy-3-(3,4,5-trimethoxybenzoyloxy-C¹⁴)-; methyl ester: 559
- 7H-Benz[o]carbazole-6-carboxylic acid, -N¹⁵: 1715
- 1,4-Benzodioxan, H²-: 1645
- 2H-1,5-Benzodioxepin, H²-3,4-dihydro-: 1645
- 1,6-Benzodioxocin, H²-2,3,4,5-tetrahydro-: 1645
- 1,3-Benzodioxole, H²-: 1645
- 11H-Benz[o]fluorene-7-carboxylic acid, 11-oxo-, -C¹⁴₁-5,6,11-C¹⁴₃: 689
- 11H-Benz[o]fluorene-11-carboxylic acid, -C¹⁴: 111
- 11H-Benz[o]fluorene-5-carboxylic acid, 11-oxo-, -C¹⁴₁-10,10a,11-C¹⁴₃; and methyl ester: 688
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- ; methyl ester: 852
- 11H-Benz[o]fluorene-11-methanol, - α -C¹⁴: 852
- 11H-Benz[o]fluorene-11-one, -11-C¹⁴: 686
- , -5,6,11-C¹⁴₃: 689
- 11H-Benz[o]fluorene-11-one, -10,10a,11-C¹⁴₃: 689
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- , 7-acetamido-6,7-dihydro-1,2,3-trimethoxy-10-methoxy-C¹⁴-: 552
- , 7-acetamido-6,7-dihydro-1,3,10-trimethoxy-2-methoxy-C¹⁴-: 553
- , 7-amino-6,7-dihydro-1,2,3-trimethoxy-10-methoxy-C¹⁴; D-tartrate: 555
- Benzo[a]heptalen-10(5H)-one, 7-acetamido-6,7-dihydro-1,2,3-trimethoxy-9-methoxy-C¹⁴-: 552
- , 7-amino-6,7-dihydro-1,2,3-trimethoxy-9-methoxy-C¹⁴-; D-tartrate: 555
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- ; calcium salt: 1438
- ; ethyl ester: 1149
- ; methyl ester: 1149
- , -C¹³: 94, 148
- , -C¹⁴: 74, 76, 88, 94, 379, 627, 629, 662, 667, 673, 677, 743, 744, 830, 836, 840, 843, 885, 887, 920, 922, 925
- ; ethyl ester: 379, 831
- ; hydrazide: 627
- ; methyl ester: 74, 543, 627
- ; sodium salt: 511
- , -1-C¹⁴: 103, 322, 827
- , -C¹⁴-1,2,3,4-C¹⁴₄: 108, 420
- , -1,2-C¹⁴₂: 414
- , -C¹⁴₁-1,2-C¹⁴₂: 106
- , -2,3-C¹⁴₂: 1146
- , -1,2,3,4-C¹⁴₄: 324
- , -C¹⁴₅: 109, 323, 420, 824
- , H²-: 1277, 1597
- , -2-H²: 1523
- , -3,4,5-H²₃: 1280
- , -H²₅: 1281
- , H³-: 1687
- , -O¹⁸: 1871, 1896
- ; methyl ester: 1875, 1877
- , -O¹⁸: 1870, 1885, 1896
- ; silver salt: 1871
- , o-acetyl-: 1826
- ; 1-(p-nitrophenyl)hydrazone-2-N¹⁵: 1826
- , o-amino-, -C¹⁴: 316
- , o-amino-, -N¹⁵: 1766
- , p-amino-, -C¹⁴: 317, 319, 456
- ; 2-diethylaminoethyl ester, hydrochloride: 456
- ; ethyl ester: 457
- , C¹⁴-p-amino-: 320
- , 4-amino-3,5-dibromo-, -Br⁸²; 2-diethylaminoethyl ester: 1151
- , o-benzoyl-, -C¹⁴: 93, 849
- , o-benzoyl-C¹⁴₁-, -C¹⁴₁: 848
- , o-benzyl-, -C¹⁴: 98
- , 4-benzyloxy-3,5-dimethoxy-, -C¹⁴: 229, 325
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- ; ammonium salt: 1149
- ; barium salt: 1148
- ; ethyl ester: 1149

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 —; methyl ester: 1149
 —, *o*-bromo-, -Br⁸²: 1148, 1149
 —; ammonium salt: 1149
 —; ethyl ester: 1149
 —; methyl ester: 1149
 —, *p*-bromo-, -C¹⁴: 887
 —, *p*-bromo-, -Br⁸²: 1148, 1149
 —; ethyl ester: 1149
 —; methyl ester: 1149
 —, *p*-*t*-butyl-, -C¹⁴: 744
 —, *m*-chloro-, -O¹⁸: 1871
 —, *p*-chloro-, -C¹⁴: 97, 597, 888
 —, *p*-chloro-, -O¹⁸: 1871
 —, *p*-chloromercuri-: 1235
 —, *p*-cyclohexyloxy-, -C¹⁴: 98, 666
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 —, 3,4-dimethoxy-, -C¹⁴: 324
 —, *o*-(2,5-dimethylbenzoyl)-: 1530
 —, 3,5-dinitro; hydrazide: 1796
 —, *p*-hydrazino-: 1547
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 —, *p*-hydroxy-, -C¹⁴: 92, 325, 743
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 —; butyl ester: 1220
 —; *sec*-butyl ester: 1220
 —; 2,3-dihydroxypropyl ester: 1220
 —; ethyl ester: 1220
 —; heptyl ester: 1220
 —; hexyl ester: 1220
 —; 4-hydroxybutyl ester: 1220
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 —, *p*-isopropoxy-, -C¹⁴: 97
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 —, 2-mercapto-, -S³⁵: 1981
 —, 2-mercapto-S³⁵-: 1981, 1982
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 Butyramide, -1,4-C¹⁴₂: 502
 —, 2-cyano-2-phenyl-, -*N*-N¹⁵: 1798
 Butyranilide, 2-acetamido-4-(benzylthio)-, -S³⁵, L-: 1962
 Butyric acid: 1596
 —; methyl ester: 1267
 —, -1-C¹¹: 95
 —, -1-C¹³: 43, 95
 —; octyl ester: 805
 —, -2-C¹³: 37
 —, -3-C¹³: 52
 —; methyl ester: 52
 —; potassium salt: 52
 —, -1-C¹⁴: 95, 911
 —, -2-C¹⁴: 37, 43
 —, -3-C¹⁴; and sodium salt: 52
 —, -4-C¹⁴: 48
 —, H²:-; ethyl ester: 1643
 —, -2-H₂: 1266, 1267
 —; methyl ester: 1267
 —, -2-H₂; methyl ester: 1267
 —, -2,3-H₂; and ethyl ester: 1267
 —; sodium salt: 1267, 1268

- , -3,4- H_2^2 ; sodium salt: 1268
- , 2-acetamido-4-(benzylthio)-, - S^{35} : 1962
- , 2-acetamido-4-cyano- C^{14} -, -1- C^{14} ; ethyl ester: 266
- , 2-acetamido-3-hydroxy-, -4- C^{14} ; ethyl ester: 182
- , 4-amino-, -4- C^{14} : 264
- , 2-amino-4-(benzylthio)-, -3,4- C_2^{13} - S^{34} : 1959
- , 2-amino-4-(benzylthio)-, -3,4- C_2^{13} : 203
- , 2-amino-4-(benzylthio)-, -3,4- H_2^2 : 1303, 1305
- , 2-amino-4-(benzylthio)-, - S^{34} : 1955
- , 2-amino-4-(benzylthio)-, - S^{35} : 1954, 1955, 1956, 1957, 1958, 1962, 1964, 1971
- , 2-amino-4-(ethylthio)-, - S^{35} (also see ethionine): 1956
- , 2-amino-4-(ethylthio-1- C^{14})-: 204
—; L-: 205
- , 2-amino-3-hydroxy-, -2- C^{14} ; L-: 179
- , 2-amino-3-hydroxy-, -4- C^{14} : 183
- , 2-amino-3-hydroxy-, - N^{15} : 1748
- , 2-amino-4-mercapto-, -3,4- H_2^2 : 1306
- , 2-amino-4-mercapto-, - S^{34} : 1955
- , 2-amino-4-mercapto-, - S^{35} : 1954, 1955
- , 2-amino-3-methoxy-, -1,2- C_2^{14} : 187
- , 2-amino-3-methoxy-, - N^{15} : 1749
- , 2-amino-3-methyl-, -1- C^{14} : 189
- , 2-amino-3-methyl-, -3,4- H_2^2 : 1295
- , 2-amino-3-methyl-, -4- C^{14} : 196
- , 2-amino-4-(methylsulfinyl)-, - S^{35} ; L-: 1960
- , 2-amino-4-(methylthio)-, -3,4- C_2^{13} : 203
- , 2-amino-4-(methylthio)-, - S^{35} : 1954
- , 2-amino-4-(methylthio- C^{14})-: 199
—; D-: 199
—; L-: 198, 199
—; D- and L-, picrate: 199
- , 2-amino-4-(methylthio- C^{14} - H_2^2)-: 1303
—; L-: 199
- , 2-amino-4-(methylthio- H_2^2)-: 1302
- , 2-amino-4-phenyl-, - N^{15} : 1763
—; D- and L-: 1765
- , 2-amino-4-ureido- C^{14} ; L-: 210
- , 2-benzamido-4-(benzylthio)-, - S^{35} : 1957
—; ethyl ester: 1957
- , 2-benzamido-4-chloro-; ethyl ester: 1957
- , 2-benzamido-3-hydroxy-, -2- C^{14} ; ethyl ester: 178
- , 2-benzamido-3-hydroxy-, - N^{15} ; ethyl ester: 1748
- , 2-benzenesulfonamido-4-(ethylthio)-: 205
- , 2-benzoyloxycarbonylamino-4-phenyl-, - N^{15} : 1764
—; D-: 1764, 1765
—; L-: 1765
—; D-, ammonium salt: 1765
—; D- and L-, α -methylbenzylamine salt: 1764
- , 2-bromo-, -1- C^{14} : 188
—; isobutyl ester: 188
- , 4-bromo-; ethyl ester: 313
- , 2-bromo-3-hydroxy-, -1- C^{14} : 188
- , 2-bromo-3-methoxy-: 1749
- , 2-bromo-3-methoxy-, -1,2- C_2^{14} : 187
- , 2-bromo-3-methyl-, -4- C^{13} : 195
- , 4-chloroformyl-; methyl ester: 64, 65
- , 4-cyano- C^{14} ; ethyl ester: 313
- , 2-cyano-3-cyano- C^{14} -3-methyl-; potassium salt: 70
- , 2-cyano-3-methyl-, -3- C^{14} ; ethyl ester: 55
- , 2-cyano-3-methyl- C^{13} -, -4- C^{13} ; ethyl ester: 55
- , 4-cyano-4- C^{14} -3-methyl-; potassium salt: 112
- , 2,4-diamino-; L-, copper complex: 210
—; L-, dihydrochloride: 210
- , 2,3-dioxo-, -3- C^{14} ; ethyl ester: 139
—; monophenylhydrazone: 140
- , 4,4'-dithiobis[2-amino-, -3,4- H_2^2]: 1306
—, 4,4'-dithio- S_2^{35} -bis[2-amino-: 1971, 1972
—, 2-ethyl-; ethyl ester: 1429, 1486
—, 2-ethyl-, -2- H^2 : 1430
—; ethyl ester: 1429, 1486
—, 4-(ethylthio)-, -1- C^{14} : 98, 159
—, 2-formamido-3-methoxy-, -1,2- C_2^{14} : 187
- , 3-hydroxy-; β -lactone: 1865
- , 3-hydroxy-, -3- O^{18} ; potassium salt: 1865
- , 3-hydroxy-, -1,3- O_2^{18} ; potassium salt: 1865
- , 4-hydroxy-; γ -lactone: 1874
- , O_2^{18} -4-hydroxy-: 1875
- , 3-hydroxy-3-methyl-, -3- C^{14} ; ethyl ester: 58
—; hydrazide: 58
—; sodium salt: 58
- , 4-hydroxy-3-methyl-; γ -lactone: 112
- , 3-hydroxy-2-thiobenzamido-, -1- C^{14} ; ethyl ester: 208

- Butyric acid (*Continued*)
 —, 3-methoxycarbonyl-3-methyl-: 71
 —, 3-methoxycarbonyl-C¹⁴-3-methyl-: 70, 72
 —, 4-(*m*-methoxyphenyl)-: 1145
 —, 4-(*p*-methoxyphenyl)-, -1-C¹⁴: 97, 546
 —, 2-methyl-, -1-C¹⁴: 96
 —; *p*-bromophenacyl ester: 91
 —, 2-methyl-, -3-C¹⁴; and ethyl ester: 54
 —, 2-methyl-, -2-H²; and methyl ester: 1325
 —, 2-methyl-C¹⁴-: 51
 —; *p*-bromophenacyl ester: 52
 —, 3-methyl-: 72
 —, 3-methyl-, -4-C¹³: 195
 —, 3-methyl-, -1-C¹⁴: 96
 —; cetyl ester: 213
 —; ethyl ester: 808
 —; sodium salt: 43, 213
 —, 3-methyl-, -3-C¹⁴: 55
 —, 3-methyl-C¹³-, -4-C¹³: 55
 —, 3-methyl-4-phenyl-, -1-C¹⁴: 97, 1143
 —, 4-phenyl-: 92
 —, 4-phenyl-, -1-C¹⁴: 97, 547
 —, 4,4'-thiobis(2-amino-,)-S³⁵: 1965
 —, 4-*p*-tolyl-, -1-C¹⁴: 79, 97, 1145
 Butyric acid-H²: 1268
 —, -2-H²: 1266
 Butyric anhydride: 1136
 β-Butyrolactone: 1865
 γ-Butyrolactone: 1874
 —, 3-methyl-: 112
 Butyronitrile: 646
 —, -C¹³: 805
 —, 2-amino-3-methyl-, -1-C¹⁴: 189
 —, 4-chloro-: 505
 —, 4-chloro-, -1-C¹⁴: 269, 451
 Butyrophenone, -3,4-H²: 1520
 —; semicarbazone: 1520
 —, 3,3-dimethyl-2-oxa-, -1-O¹⁸: 1886
 —, 2-(*p*-methoxyphenyl)-4-methoxy-: 958
 —, H²-2-methyl-, L-: 1605
 —, 3-methyl-2-oxa-, -1-O¹⁸: 1885
 —, 2-oxa-, -1-O¹⁸: 1885
 Butyryl chloride: 1268
 —, 4-benzyloxycarbonyl-4-benzyloxy-carbonylamino-: 1759
 —, 4-carbomethoxy-: 64, 65
 —, 4-(*m*-methoxyphenyl)-: 1145
 —, 3-methyl-, -1-C¹⁴: 93
 —, 4-*p*-tolyl-, -1-C¹⁴: 1145
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- Cadaverine, -1,5-C¹⁴₂; and dipicrate: 280
- Cadmium, bis(ethyl-1-C¹⁴)-: 65
 —, bis(methyl-H³)-: 1569
 —, bis(7-methyl-4-indanyl)-: 853
 —, bis(phenyl-C¹⁴₄)-: 926
 —, dihexyl-, -1-C¹⁴: 65
 —, dimethyl-: 663
 —, dimethyl-, -C¹⁴: 1073, 1074
 —, di-1-naphthyl-: 855
 —, diphenyl-: 664, 923, 925
 —, dipropyl-, -2-C¹⁴: 65
 —, ditridecyl-, -1-C¹⁴: 65
 Caffeine, C¹⁴-: 767
 Camphane, H²-: 1617
 —, -2-H²: 1583
 —, -2,3-H²: 1584
 —, 2-chloro-: 1583
 Camphene; hydrochloride-Cl³⁸: 1192
 —, -8-C¹⁴: 818, 819
 —; hydrochloride: 819
 —, -8,9,10-C¹⁴₃: 819
 —, H²-; hydrochloride-Cl³⁸: 1192
 Camphenilone: 818, 819
 —; semicarbazone: 818
 —, -8,9-C¹⁴₂: 819
 Camphoric acid; D- and L-: 1789
 Camphorsulfonic acid, bromo-; D-, ammonium salt: 1374
 Capric acid, -C¹⁴: 96
 Caprylic acid; methyl ester: 1431
 Caproic acid, -C¹⁴: 96
 Carbamic acid; 2-chloro-1-(chloro-methyl)ethyl ester: 603
 —, -C¹⁴; ammonium salt: 595
 —; ethyl ester: 602
 —, *N,N*-diethyl-, -C¹⁴; 2-chloro-1-(chloromethyl)ethyl ester: 602
 —, diethyldithio-, -S³⁵; potassium salt: 1994
 —, ethylene-C¹⁴-di-; ethyl ester: 496
 Carbamoyl chloride: 1280
 —, diethyl-: 404, 405
 Carbanilic acid, *p*-phenyl-; (+)-α-ethyl-1,2-H²-benzyl ester: 1313
 —; (+)-α-vinylbenzyl ester: 1313
 Carbanilide: 390
 —, 4,4'-dichloro-: 601
 —, thio-: 161
 Carbanilonitrile, *N*-methyl-*p*-nitro-, N¹⁵: 1720
 9-Carbazolemethanol, 3,6-dichloro-: 616
 Carbide, aluminum: 1402, 1679
 —, barium-, -C¹⁴: 45, 912
 —, calcium: 1433, 1706
 —, magnesium: 1427, 1435, 1436
 Carbon-C¹⁴ black: 561, 562, 570
 Carbon dioxide: 1402
 —, -C¹¹: 33, 117, 568
 —, -C¹³: 95, 578, 579

- , $-C^{14}$: 32, 33, 35, 36, 56, 61, 63, 67, 72, 79, 88, 95, 109, 110, 116, 117, 125, 159, 274, 316, 318, 324, 365, 366, 373, 392, 432, 435, 477, 504, 561, 562, 565, 566, 570, 576, 580, 582, 595, 607, 791, 801, 820, 899, 902, 1089, 1143, 1339
- Carbon dioxide- O_2^{18} : 1871, 1883, 1886, 1894
- Carbon disulfide: 207, 1861
- Carbon disulfide- S_2^{35} : 1991, 1992, 1994
- Carbonic acid; ammonium salt: 1954
- , 2-carboxy- C^{14} -3-methyl-1,5-phenylene; dimethyl ester: 371
- ; diethyl ester: 574
- , 2-formyl- C^{14} -3-methyl-1,5-phenylene; dimethyl ester: 371
- ; diphenyl ester: 1857, 1858
- , $-C^{13}$; barium salt: 579
- ; calcium salt: 576
- , $-C^{14}$; ammonium salt: 370
- ; barium salt: 42, 45, 272, 563, 564, 581, 587, 588, 592
- ; diethyl ester: 444, 483, 484
- ; dipotassium salt: 32, 117
- ; disilver salt: 444, 483
- ; disodium salt: 31, 32
- , chloro-; ethyl ester: 1719, 1983
- Carbon monoxide: 909, 1338, 1359, 1402, 1512, 2008, 2009
- , $-C^{13}$: 576, 577
- , $-C^{14}$: 122, 576, 578, 586, 1049
- Carbon suboxide: 1262, 1263
- Carbon tetrabromide, $-C^{14}$: 649, 918
- Carbon tetrabromide- Br_2^{80} : 1167
- Carbon tetrabromide- Br_2^{82} : 1167
- Carbon tetrachloride: 1402, 1991
- , $-C^{14}$: 864
- Carbon tetrachloride- Cl_2^{36} : 1190
- Carotene, -15,15'- C_2^{14} ; β -, all-*trans*-: 1113
- ; β -, 15,15'-mono-*cis*-: 1113
- , 15,15'-dehydro-, -15,15'- C_2^{14} ; β -: 1113
- Casein: 1217
- , I^{131} -Iodo-: 1217, 1219
- Catechol: 1350
- ; diisobutyl ether: 1645
- , $-H_2^2$: 1350
- Cetyl alcohol: 2005
- Cetyl bromide: 213
- Cellulose, H^2 -: 1642
- Chalcone, $-C^{14}$: 669
- , 4-methoxy-, $-C^{14}$: 671
- , 4'-methoxy-, $-C^{14}$: 643, 671
- , β -(1-naphthyl)-: 1462
- , β -phenyl- H_2^2 -: 1342
- Chavicol, 2-allyl-1- C_1^{14} -6-allyl-, $-\gamma-C_1^{14}$: 740
- , 2,6-dimethyl-, $-\gamma-C^{14}$: 735
- , 2,6-dimethyl-, $-\alpha, \gamma-C_1^{14}$: 737
- Chelidonic acid- H_2^2 , $-H_2^2$: 1388
- Chenodeoxycholic acid, -24- C^{14} : 1059
- Chiniofon-, $-I^{131}$: 1238
- Chloral: 1230, 1488, 1490
- ; hydrate: 892
- ; hydrate- H_2^2 : 1488
- , -1- C^{14} : 889
- Chloramine-T: 48
- Chloramphenicol, C_1^{14} -: 385
- Chloranil: 925
- Chloretone, C_2^{14} -: 956
- Chlorine, (exchange reactions): 1190
- , $-Cl_2^{36}$: 1178, 1179, 1180, 1182, 1193, 1194
- Chloroform: 1359, 1401
- , $-C^{13}$: 875
- , $-C^{14}$: 872, 874
- , $-Cl_2^{36}$: 1190
- , $-H^2$: 1359, 1488, 1489, 1490, 1607
- Chloroformal; *p*-nitrophenylhydrazone: 1721
- Chlorosulfonic acid, $-S^{35}$: 2006
- Chlorpromazine, C_1^{14} -: 532
- 22(22),23-Choladien-3 α -ol, 24,24-diphenyl-, -11,12- H_2^2 ; acetate: 1565
- 3 α ,24-Cholanediol, 24,24-diphenyl-, -11,12- H_2^2 : 1565
- Cholanic acid, -24- C^{14} : 1059
- , 3 α -hydroxy-, -24- C^{14} : 1059
- , 3 α -hydroxy-, -11,12- H_2^2 : 1564
- ; methyl ester: 1564, 1565, 1567
- , 3 α -hydroxy-, -3 β ,4,5- H_2^2 : 1537
- , 3 α -hydroxy-, -11,12- H_2^2 ; methyl ester, acetate: 1693, 1694
- , H^2 -3 α -hydroxy-; methyl ester, acetate: 1567
- , 3 β -hydroxy-, -3 α ,4,5- H_2^2 : 1538
- , 3-oxo-, -4,5- H_2^2 ; methyl ester: 1538
- Cholanthrene, 3-methyl-; picrate: 856
- , 3-methyl-, -6- C^{14} : 853, 855
- , 20-methyl-, -11- C^{14} : 855
- 4-Cholenic acid, 3-oxo-; methyl ester: 1537, 1538
- 5-Cholenic acid, C^{14} -3 β -hydroxy-: 1065
- , H^2 -3 β -hydroxy-; methyl ester, acetate: 1536
- 9(11)-Cholenic acid, 3 α -hydroxy-; methyl ester, acetate: 1567
- 11-Cholenic acid, 3 α -hydroxy-; methyl ester, acetate: 1564, 1693
- 23-Cholen-3 α -ol, 22-bromo-24,24-diphenyl-, -11,12- H_2^2 ; acetate: 1565

23-Cholen-3 α -ol (*Continued*)

—, 24,24-diphenyl-, -11,12-H₂²; and acetate: 1565

3,5-Cholestadiene, -4-C¹⁴: 1053

3,5-Cholestadien-3 β -ol, -3-C¹⁴; acetate: 1055

—, -4-C¹⁴; acetate: 1052, 1053

4,6-Cholestadien-3 β -ol, -4-C¹⁴; and benzoate: 1135

5,7-Cholestadien-3 β -ol: 1236

—; *p*-iodobenzoate-I¹³¹: 1236

—, -3-C¹⁴: 1129

—; acetate: 1130

—; 3,5-dinitrobenzoate: 1130

—, -4-C¹⁴: 1135

—; benzoate: 1135

—; 3,5-dinitrobenzoate: 1134

5,25-Cholestadien-3 β -ol, -26,27-C¹⁴₂; acetate: 1057

6,8-Cholestadien-3 β -ol; acetate-H₂²: 1545

Cholestane, C¹⁴-.: 1106

—, H²-.: 1548, 1551

—, -3 α -H²: 1582

—, -3 β -H²: 1582

—, -5,6,7-H₂²: 1550

3 β ,7-Cholestanediol, -6 β -H²; 3-acetate: 1582

3 α -Cholestanol, -3 β -H²: 1581

—, -2-H₁²: 1581

3 β -Cholestanol: 1236

—; *p*-iodobenzoate-I¹³¹: 1236

—; *p*-toluenesulfonate: 1582

—, C¹⁴-.: 1105, 1106

—, -3 α -H²: 1546, 1580

—; *p*-toluenesulfonate; 1582

—, -2-H₁²: 1581

—, -5,6,7-H₂²; and acetate: 1580

—, H²-.: 1579, 1580, 1695

—, -5,6-H₂²: 1695

—, 5,6-dibromo-.: 1695

—, C¹⁴-5,6-dibromo-; acetate: 1081

—, H²-5,6-dibromo-.: 1547

—, 6 α ,7 α -epoxy-; acetate: 1582

3-Cholestanone: 1581

—, C¹⁴-.; and hydrazone: 1106

—, H²-.: 1536, 1547, 1579, 1580

—, -2-H₁²: 1581

—, -2,4-H₂²: 1555

—, -5,6,7-H₂²: 1580

7-Cholestanone, H₂²-.: 1555

—, 3 β -hydroxy-, -6 β -H²; acetate: 1582

2,4,6-Cholestatriene, -4-C¹⁴: 1135

2-Cholestene: 1551

5-Cholestene: 1551

—, C¹⁴-.: 1103, 1105, 1107

—, -3 β -H²: 1582

—, C¹⁴-3 β -chloro-.: 1102

5-Cholestene-3 β ,7-diol, -4-C¹⁴: 1055

5-Cholestene-3 β ,17 β -diol, 7,7, -(ethylenedithio); 3-acetate, 17-benzoate: 1543

5-Cholestene-3 β ,25-diol, -26-C¹⁴; and 3-acetate: 1057

5-Cholestene-3 β ,5 α ,6 β -triol, -4-C¹⁴: 1055

2-Cholesten-3 β -ol; acetate: 1580

4-Cholesten-3 α -ol, -4-C¹⁴: 1055

4-Cholesten-3 β -ol, -4-C¹⁴: 1055

5-Cholesten-3 α -ol, 3-methyl-C¹⁴-4-oxa-.: 1100

5-Cholesten-3 β -ol, -5,6,7-H₂²; acetate: 1580

—, 7,7-(ethylenedithio)-; acetate: 1543

Cholesten-3 β -ol, H³-.: 1695

4-Cholesten-3-one: 1537, 1583

—, -3-C¹⁴: 1055, 1097, 1099, 1101

—; 3-enol acetate: 1055

—, -4-C¹⁴: 1053, 1055, 1099, 1100, 1101

—; 3-enol acetate: 1052, 1053, 1055

—, H²-.: 1534, 1536, 1547, 1549

—; 3-enol acetate: 1549

—, -7-H₁²: 1542

5-Cholesten-3-one: 1054, 1546

5-Cholesten-7-one, C¹⁴-.: 1103

3 β -hydroxy-, -4-C¹⁴: 1055

Cholesterol: 1236, 1696

—; acetate: 1694

—; acetate-H₂²: 1545

—; *p*-iodobenzoate-I¹³¹: 1236

—; *p*-toluenesulfonate: 1582

—, C¹⁴-.: 1081, 1102, 1105

—, -3-C¹⁴: 1055

—; acetate: 1055, 1129

—, -4-C¹⁴: 1052, 1053, 1055

—; benzoate: 1055, 1134

—, -6-C¹⁴: 1058

—, -26-C¹⁴: 1058

—, H²-.: 1534, 1547, 1549, 1579, 1580

—; acetate: 1535, 1537

—, -3-H²: 1546

—, -7-H₁²: 1542, 1544

—, -7-H₂²: 1543

—, H³-.: 1694, 1696

—; acetate: 1694

—, 7-bromo-; benzoate: 1542, 1544

—, 7-bromo-, -3-C¹⁴; acetate: 1130

—, 7-bromo-, -4-C¹⁴; benzoate: 1134

—, 7-dehydro-.: 1236

—; acetate: 1695

—; *p*-iodobenzoate-I¹³¹: 1236

—, 7-dehydro-, -3-C¹⁴: 1129

—; acetate: 1130

—, 7-dehydro-, -4-C¹⁴; 3,5-dinitrobenzoate: 1134

—, 25-dehydro-, -26-C¹⁴: 1058

- , 25-dehydro-, -26,27- C_{14}^{14} ; acetate: 1057
- , 5 α ,6 β -dihydroxy-, -4- C_{14}^{14} : 1055
- , 7-hydroxy-, -4- C_{14}^{14} : 1055
- , 25-hydroxy-, -26- C_{14}^{14} : 1057
- , 7-oxo-, -4- C_{14}^{14} : 1055
- Cholesteryl chloride, C_{14}^{14} : 1102
- Cholic acid: 1539
- , -4- C_{14}^{14} : 72
- , -24- C_{14}^{14} : 1059
- , H^2 :-: 1539
- ; methyl ester: 1540
- Choline, - N^{15} : 1836
- Choline acetate, C_{14}^{14} :-: 962
- Choline bromide, C_{14}^{14} :-: 966
- , acetyl-, - N^{15} : 1837
- , acetyl[C_{14}^{14} :-: 966
- , acetyl- H^2 :-: 1525
- Choline chloride: 962, 1909
- ; phosphate- P^{32} , calcium salt: 1909
- , C_{14}^{14} :-: 961, 967, 969
- , - α - C_{14}^{14} : 963
- , - β - C_{14}^{14} : 965
- , H^2 :-: 1524
- , - N^{15} : 1836
- , acetyl[C_{14}^{14} :-: 967
- Choline chloroaurate, - N^{15} : 1836
- Choline chloroplatinate, C_{14}^{14} :-: 964
- Choline picrate, C_{14}^{14} :-: 962
- Choline reineckate, C_{14}^{14} :-: 961
- , H^2 :-: 1524
- Chromone, 7-hydroxy-2-phenyl-, -2- C_{14}^{14} : 700
- Chrysanthemummonocarbonyl chloride; *trans*:-: 468
- , C_{14}^{14} :-: 465
- Chromatography: 16
- Chrysanthemummonocarboxylic acid: *trans*-, 3-allyl-2-methyl- C_{14}^{14} -4-oxo-2-cyclopenten-1-yl-1- C_{14}^{14} ester: 468
- , C_{14}^{14} :-; and allethronyl ester: 465
- Chrysene, -5,6- C_{14}^{14} : 727
- 5,6-Chrysenedione, -5,6,11,12- C_{14}^{14} : 688, 727
- ; mono-oxime: 688
- 6-Chrysofluorene-carboxylic acid, 11-oxo-, - C_{14}^{14} : 11- C_{14}^{14} : 686, 687
- 7-Chrysofluorene-carboxylic acid, 11-oxo-, - C_{14}^{14} : 5,6,11- C_{14}^{14} ; and methyl ester: 689
- 11-Chrysofluorene-carboxylic acid, - C_{14}^{14} : 727; methyl ester: 727
- 11-Chrysofluorene-methanol, - α - C_{14}^{14} : 727; *p*-nitrobenzoate: 728
- 11-Chrysofluorenone, -11- C_{14}^{14} : 686, 687
- , -5,6,11- C_{14}^{14} : 689
- Cinchonine: 1778
- α -Cinenic acid, C_{14}^{14} :-: 355
- α -Cinenonitrile, C_{14}^{14} :-: 355
- Cinnamaldehyde: 1999; sodium bisulfite addition compound: 1999
- Cinnamic acid: 78, 1277
- ; ethyl ester: 1870
- , - C_{13}^{13} : 300
- , - C_{14}^{14} : 78
- ; methyl ester: 301
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- , 4-(tetra-*O*-acetyl- β -D-glucosyloxy)-3-methoxy-, - α - C_{14}^{14} : 1033
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—, *N*-benzoyl-; methyl ester: 1970

—, *S*-benzyl-, $-S^{35}_2$: 1967, 1968, 1970

—; L:- 1970

—, *S*-benzyl-*N*-formyl-, $-3-C^{14}$; and D:- 208

—, *S*-benzyl-2-methyl-, $-1-C^{14}$: 209

—, *S*-[(benzylthio)carbonyl]-, $-3-C^{14}$; hydrochloride: 207

—, *N*,*S*-diacetyl-, $-S^{35}_2$: 1966

—, *N*,*S*-dibenzoyl-: 1970

—, 3-methyl-, $-1-C^{14}$: 208

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—; D- and L:- 208

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—, $-S^{35}_2$: 1966, 1967, 1969

—; D:- 1969

—; L:- 1969, 1970

—; brucine salt: 1969

—, *N*,*N'*-bis(phenylacetyl)-, $-S^{35}_2$; L:- 1970

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—, bis(acetyl- $1-C^{14}$)-; dimethyl ester: 471

—, I^{131}_2 -diiodo-; dimethyl ester: 1246

—, monoacetyl- $1-C^{14}$;- dimethyl ester: 471

Dextran: 2006

—; sulfate- $-S^{35}_2$: 2006

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3-Dibenzothiophenamine, $-S^{35}_2$: 1980

Dibenzothiophene; 5,5-dioxide: 1979

—, $-S^{35}_2$; and 5-oxide: 1979

—, 3-amino-, $-S^{35}_2$: 1980

—, 3-nitro-, $-S^{35}_2$; 5-oxide: 1979

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 —, $-1,1'-C_2^{14}$: 406, 499
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 —, 2,2'-dichloro- N -methyl-: 1203
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 —, 5-methyl-, $-C_1^{14}$:- 683, 684
 Diphenylamine; hydrochloride: 1830
 —, H^2 :- 1630
 —, $-N^{15}$: 1727, 1833
 —, N -nitroso- N^{15} :- 1830
 Diphenylene, $-N_2^{15}$; sulfate: 1723
 Distyrene, H^2 :- 1639
 Disulfide, 2,2'-bis(benzothiazolyl)-, $-S_2^{35}$: 1982
 —, bis(diethylthiocarbamoyl)- $-S_2^{35}$, $-S_2^{35}$: 1994
 —, tetraethylthiuram: 1994
 —, tetraethylthiuram- $-S_2^{35}$, $-S_2^{35}$: 1994
 Docosane, -11,12- C_2^{14} : 64
 Dodecane, -1,2- H_2^3 : 1417
 —, 1-bromo-: 96, 1934
 —, 1-bromo-, -1- C^{14} : 65, 542, 970
 Dodecanenitrile, -1- C^{14} : 508
 Dodecanethiol, $-S^{35}$: 1934
 Dodecanoic acid, -1- C^{14} : 60
 —; glyceryl ester: 61
 —; methyl ester: 65, 882
 Dodecanoyl chloride, 12-carbomethoxy-: 65
 Dodecylamine, -1- C^{14} : 508
 Dodecyl alcohol: 805
 —, -1- C^{14} : 882
 Dodecyl mercaptan- $-S^{35}$: 1934
 Durene, H^2 :- 1638

Durohydroquinone, $-\alpha-C^{14}$: 720, 721
 Duroquinone, $-\alpha-C^{14}$: 720, 721

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Elaidic acid: 1666
 Epiandrosterone, C^{14} :- 1105
 —, dehydro-: 1085
 —, dehydro-, -16- C^{13} ; acetate: 1096
 Epichlorohydrin: 242
 —, $-Cl^{36}$: 1183
 Epicholesterol, -4- C^{14} : 1053, 1056
 —, -3- H^2 : 1546
 3 β -Ergostanol; acetate- H_3^3 : 1545
 14-Ergosten-3 β -ol; acetate- H_3^3 : 1545
 8(14)-Ergosten-3-one, -2,4- H_4^3 : 1555
 Ergosterol, H^2 :- 1549
 Erythromycin, C_1^{14} :- 952
 —, de- N -methyl-: 952, 953
 —, O,N -dicarbobenzyloxy-de- N -methyl-: 953
 —, N -methyl- C^{14} -de- N -methyl-: 952
 Erythrose; D-: 974, 977, 978
 —; L-: 975, 977
 —; L-, diacetamide- $-N_2^{15}$: 1862
 —, 1,1-diacetamido-1-deoxy-, $-N_2^{15}$; L-: 1862
 —, 2,4- O -ethylidine-; D-: 977
 Estradiol: 1237
 —, -16- C^{14} : 1091, 1092
 —; 17-acetate, 3-methyl ether: 1092
 —; diacetate: 1092
 —; dipropionate: 1092
 —; 3-methyl ether: 1090
 —, 2,4-diiodo-, $-I_2^{31}$: 1236
 —, iodo-, $-I^{31}$: 1237
 —, 17 α -methyl- C^{14} :- 1093
 —; monobenzoate: 1093
 Estragole, 2-allyl- $-C_1^{14}$ -6-allyl-, $-\gamma-C_1^{14}$:- 740
 —, 2,6-dimethyl-, $-\alpha, \gamma-C_1^{14}$:- 738
 —, 3,5-dimethyl-, $-\gamma-C^{14}$: 735
o-Estragole, $-\alpha-C^{14}$: 736
 —, 6-allyl-, $-\alpha-C^{14}$: 740
 —, 4-methyl-, $-\alpha-C^{14}$: 623
 1,3,5(10),6-Estratetraen-17-one, 3 β -acetoxy-: 1562
 1,3,5(10)-Estratrien-17 β -ol, 16,16-bis-(ethylthio)-3-methoxy-, -16- C^{14} ; acetate: 1090
 5,7,9-Estratrien-17 β -ol: 1545
 —; acetate- H_3^3 : 1545
 1,3,5(10)-Estratrien-16-one, 17 β -hydroxy-3-methoxy-, -16- C^{14} ; and acetate: 1090
 1,3,5(10)-Estratrien-17-one, 3 β -acetoxy-, -6,7- H_2^3 : 1562

Estrone: 1094, 1561

—; acetate: 1093

—; acetate- H_3^1 : 1545

—; methyl- C^{14} ether: 1094

Estrone, -16- C^{14} : 1092

—, H^2 :- 1561

—, -6,7- H_2^1 ; acetate: 1562

—, 6-dehydro-; acetate: 1562

—, 7,8-dibromo-, - Br_2^{82} : 1162

Ethane: 1407

—, - C_2^{14} : 644, 806, 867

—, H^2 :- 1609

—, - H_1^1 : 1403, 1407

—, H_2^1 :- 1408

—, -1- H_2^1 : 1404, 1422

—, -1,2- H_2^1 : 1406

—, H_3^1 :- 1408

—, -1- H_3^1 : 1404, 1405

—, H_4^1 :- 1408

—, -1,1,2,2- H_2^1 : 1405, 1406

—, - H_2^1 : 1407, 1408

—, - H_2^1 : 1399, 1407, 1408

—, -1- $H_2^1/1$ -1- $H_3^1/4$: 1412

—, bromo-: 95, 501, 663, 805, 1231, 1513, 1583, 1883

—, bromo-, - Br^{80} : 1156, 1169

—, bromo-, - Br^{82} : 1169

—, 1-bromo-, -1- C^{14} : 37, 653, 909, 910

—, 1-bromo-, -2- C^{14} : 37, 653, 906, 909

—, 1-bromo-, -1- H_1^1 : 1471

—, 1-bromo-, -2- H_1^1 : 1471, 1472

—, 1-bromo-, -1- H_2^1 : 1473, 1498

—, 1-bromo-, -2- H_2^1 : 1473

—, bromo-, - H_2^1 : 1318, 1347, 1473, 1474, 1515

—, C_1^{13} -bromo-: 915

—, H^2 -bromo-: 1607

—, 1-bromo-2-chloro-: 203

—, 2-bromo-1,1,2-trichloro-, -1- H^2 : 1491

—, chloro-: 95, 836

—, 1-chloro-, -2- C^{14} : 835, 866

—, chloro-, - C_1^{14} : 836, 866

—, chloro-, - Cl^{36} : 1190

—, 1,1-dibromo-: 1404

—, 1,2-dibromo-: 126, 129, 1149, 1151, 1165, 1836

—, 1,2-dibromo-, - Br_2^{80} : 1169

—, 1,2-dibromo-, - Br_2^{82} : 1169

—, 1,2-dibromo-, - C_2^{13} : 130

—, 1,2-dibromo-, - C_1^{14} : 495

—, 1,2-dibromo-, - C_2^{14} : 130, 133

—, 1,2-dibromo-, - H_1^1 : 1475, 1477

—, 1,2-dibromo-, -1- H_2^1 : 1475, 1477

—, 1,2-dibromo-, -1,2- H_2^1 : 1304, 1357, 1424, 1475, 1477, 1479

—; *meso* and DL-: 1423

—, 1,2-dibromo-, - H_3^1 : 1475, 1477

—, 1,2-dibromo-, - H_2^1 : 1332, 1355, 1381, 1425, 1426, 1475, 1477, 1480, 1481

—, 1,2-dichloro-: 130, 1306, 1993

—, 1,2-dichloro-, - C_1^{13} : 201, 1993

—, 1,2-dichloro-, - H_2^1 : 1490

—, 1,2-diiodo-, - I_2^{31} : 1228

—, I^{131} -1,2-diiodo-: 1253

—, 1,1-dimethoxy-2-phenyl-: 219

—, 1,1-diphenyl-, -1- H^2 : 1451

—, H^2 -1,2-diphenyl-: 1639

—, 1,2-diphthalimido-, - C_1^{14} : 495

—, 1,2-epoxy-, - H_2^1 (also see Oxirane-, H_2^1): 1381

—, hexachloro-, - C_2^{14} : 865

—, H^2 -hexaphenyl-: 1639

—, iodo-: 95, 438, 483, 963, 1156, 1403, 1798, 1904, 1956, 2001

—, 1-iodo-, -1- C^{13} : 37, 909, 915

—, 1-iodo-, -1- C^{13} -2- C^{14} : 43, 141, 909

—, 1-iodo-, -2- C^{13} : 37, 43, 909

—, iodo-, - C_2^{13} : 868, 915

—, 1-iodo-, -1- C^{14} : 37, 43, 52, 53, 204, 205, 477, 729, 870, 871, 877, 905, 910, 958

—, 1-iodo-, -2- C^{14} : 828

—, iodo-, - C_2^{14} : 828, 867, 868

—, 1-iodo-, -2- H_1^1 : 1472, 1505

—, 1-iodo-, -1- H_2^1 : 1318

—, 1-iodo-, -2- H_2^1 : 1318

—, iodo-, - H_2^1 : 1318, 1331

—, iodo-, - I^{128} : 1227, 1253

—, iodo-, - I^{131} : 1228, 1253

—, nitro-: 1359, 1369, 1507

—, 1-nitro-, -1- H_1^1 : 1362, 1507

—, 1-nitro-, -1- H_2^1 : 1359

—, 1-nitro-, -2- H_2^1 : 1507

—, 1,1,1,2-tetrachloro-, - H_2^1 : 1492

—, 1,1,2,2-tetrachloro-, - H_2^1 : 1492

—, 1,1,2-tribromo-, - Br_3^{82} : 1168

—, 1,1,2-tribromo-, -1- H^2 : 1476, 1477

—, 1,1,2-tribromo-, -2- H_2^1 : 1476, 1477

—, 1,1,2-tribromo-, -2- H_2^1 : 1476, 1477

—, 1,1,2-tribromo-, - H_3^1 : 1476, 1477

—, 1,1,1-trichloro-: 1405

—, 1,1,1-trichloro-2,2-bis(*p*-bromophenyl)-, - Br_2^{82} : 1158

—, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)-, -2- C^{14} : 888, 889

—, 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl-4- C^{14})-: 892

—, 1,1,1-trichloro-2,2-bis(*p*-iodophenyl)-, - I_2^{31} : 1229

—, 1,1,1-trichloro-2-(*o*-chlorophenyl)-2-(*p*-chlorophenyl)-, -2- C^{14} : 890

—, 1,1,1-trichloro-2-(*o*-iodophenyl)-2-(*p*-iodophenyl)-, - I_2^{31} : 1230

—, 1,1,1-trifluoro-2-iodo-, - H_2^1 : 1502

—, 1,1,1-trifluoro-2-iodo-, - H_2^1 : 1502

Ethane (*Continued*)

- , 1,1,2-triphenyl-, -C₁₄: 925
- 1,2-Ethanediol, -C₁₄: 778
- ; dibenzoate: 779
- , -1,2-H₂; and diacetate: 1357
- , -1,2-H₂: 1355, 1358
- ; diacetate: 1355
- ; dinitrate ester: 1332
- , 1,1-bis(phenyl-C₁₄)-2-phenyl-: 675
- , 1,2-diphenyl-, -1,2-H₂: 1365
- , 1-phenyl-C₁₄, 1,2-diphenyl-: 676
- , tetraphenyl-, -1-C₁₄: 677
- , 1,1,2-triphenyl-, -1-C₁₄: 676
- , 1,1,2-triphenyl-, -2-C₁₄: 677, 928
- , 1,2,2-triphenyl-, -1-C₁₄: 928
- Ethanesulfonic acid, -S³⁵; ethyl ester: 2001
- , 2-amino-, -S³⁵: 2000
- 1,1,2,2-Ethanetetra-carboxylic acid; tetraethyl ester: 1316
- Ethanthiol: 1090
- ; sodium derivative: 158, 1341
- , H²-.: 1602
- , -H²: 1341
- , 2-amino-, -S³⁵: 1938
- , 2-diethylamino-, -S³⁵: 1976
- , 2-(ethylthio)-; sodium derivative: 1923
- 9,10-Ethanoanthracene-11-carboxylic acid, 9,10-dihydro; methyl ester: 1320, 1321
- 9,10-Ethanoanthracene-11-methanol, 9,10-dihydro-, -α-H₂: 1320
- ; acetate: 1321
- Ethanol: 691, 813, 1227, 1228, 1795, 1914
- ; aluminum derivative: 1340
- ; nitrite ester: 452
- ; nitrite-N¹⁵ester: 1721, 1795
- ; phosphate ester: 415, 416, 1927
- ; sulfate ester: 37, 417, 500, 1883, 1890
- ; sulfite ester: 1340
- ; sodium derivative: 1340
- , -1-C¹³: 909, 915
- , -1-C¹³-2-C¹⁴: 909
- , -2-C¹³: 909
- , -2-C¹³-1-C¹⁴: 618, 909
- , -C₂¹³: 868, 915
- , -1-C¹⁴: 495, 619, 870, 871, 889, 905, 908
- ; hexakis tetrapolyphosphate ester: 477
- ; phosphate ester: 477
- , -2-C¹⁴: 619, 866, 906, 907, 909, 910
- , -C₂¹⁴: 913, 916
- , -C_{1/2}¹⁴: 501, 915
- ; 3,5-dinitrobenzoate: 915
- , H²-.: 1601
- , -H²: 1340, 1349, 1401, 1402, 1471
- , -H²-1-H₁: 1471
- , -1-H₁: 1471
- , -1-H₂: 1341, 1363, 1473
- ; acetate: 1319
- , -2-H₂; acetate: 1319
- , -H₂; acetate: 1319
- ; nitrate ester: 1331
- , -H₂: 1331, 1474
- , -1-H₂: 1669
- , -O¹⁸: 1883, 1890
- , 1-amino-, -2-C¹³-1-C¹⁴: 618
- , 2-amino-: 1354, 1524
- , 2-amino-, -1-C_{1/1}¹³-2-C_{1/1}¹⁴; hydrochloride: 947
- , 2-amino-, -1-C_{1/1}¹³-2-C_{1/1}¹⁴-1-H₂; and hydrochloride: 1353
- , 2-amino-, -1-C_{1/1}¹³-2-C_{1/1}¹⁴-2-H₂; hydrochloride: 948
- , 2-amino-, -2-C¹⁴; hydrochloride: 945
- , 2-amino-, -C₂¹⁴; hydrochloride: 408, 409, 946
- , 2-amino-, -C₂¹⁴; hydrochloride: 948
- , 2-amino-, -1,2-H₂; and hydrochloride: 1707
- , 2-amino-, -N¹⁵; and hydrochloride: 1836
- , 2-[benzyl-α-C¹⁴-(1-methyl-2-phenoxyethyl) amino]-: 525
- , 2-(benzylthio)-, -S³⁵: 1937, 1993
- , 2-(4-biphenyl)-2-phenyl-, -1-C¹⁴: 743
- , 1,2-bis(phenyl-C₁₄)-2-phenyl-: 420, 926
- ; acetate: 420, 926
- ; formate: 842
- , 2-bromo-: 1525
- ; acetate: 1837
- , 2-bromo-, -C₂¹⁴: 915, 966
- , 2-bromo-1,1,2-triphenyl-, -1-C¹⁴: 926
- , 2-(*p*-*t*-butylphenyl)-2-phenyl-, -1-C¹⁴: 742
- , 2-(butylthio)-, -S³⁵: 1992
- , 2-chloro-: 43, 257, 731, 1836, 1939, 1992
- , 2-chloro-, -2-C¹⁴: 955, 963
- , 2-chloro-, -C₂¹⁴: 913, 946
- , 2-chloro-, -1,2-H₂: 1381
- , 2-chloro-, -1,2-H₂: 1706
- , 1-(*p*-chlorophenyl)-2,2,2-trichloro-, -1-C¹⁴: 888
- ; acetate: 890
- , 2-(*p*-cumenyl)-2-phenyl-, -1-C¹⁴: 742
- , 2-diethylamino-: 457
- , 1,1-dimethyl-, -O¹⁸; chromate: 1873
- , 2-dimethylamino-: 524, 961

- , 2-dimethylamino-, -1-C¹⁴: 965
- , 2-dimethylamino-C¹⁴-: 948
- ; picrolonate: 949
- , 2-dimethylamino-1,1'-H₂-: 1354
- , 1,2-diphenyl-, -2-H₂; *erythro*: 1460, 1461
- ; *threo*: 1459, 1461
- ; *erythro* and *threo*, acetate: 1460
- ; *erythro* and *threo*, benzoate: 1460
- ; *erythro* and *threo*, 2,4,6-triethylbenzoate: 1460
- , 2,2-diphenyl-, -1-C¹⁴: 744
- , 2-(*p*-ethylphenyl)-2-phenyl-, -1-C¹⁴: 742
- ; 1-naphthylcarbamate: 744
- , 2,2'-iminodi-, -C¹⁴: 946
- , 2-mesityl-2-phenyl-, -1-C¹⁴: 743
- ; 1-naphthylcarbamate: 744
- , 2-(*p*-methoxyphenyl)-2-phenyl-, -1-C¹⁴: 742
- , 1-methyl-, -1-C¹⁴ (also see 2-Propanol): 37
- ; carbanilate: 604
- ; *N*-phenylcarbamate: 604
- , 2-methylamino-: 948
- , 2-methyl-H₂-amino-; hydrochloride: 1354
- ; picrate: 1354
- , 2,2'-(methylimino-C¹⁴) di-: 517
- , *N*-methyl-C¹⁴-2,2'-iminodi-: 517
- , 2-(1-methyl-2-phenoxyethylamino)-: 525
- , 2-nitro-, -1-C¹⁴: 422
- , 2-phenyl-, -C¹⁴₂: 919
- , 1-phenyl-C¹⁴₄-1,2-diphenyl-: 841
- , 1-phenyl-C¹⁴₄-2,2-diphenyl-: 419, 841, 926
- ; acetate: 419
- ; *p*-toluenesulfonate: 926
- , 2-phenyl-2-(*m*-tolyl)-, -1-C¹⁴: 743
- ; 1-naphthylcarbamate: 744
- , 2-phenyl-2-(*o*-tolyl)-, -1-C¹⁴: 743
- , 2-phenyl-2-(*p*-tolyl)-, -1-C¹⁴: 742
- ; *p*-toluenesulfonate: 430
- , 2-phenyl-2-(3,4-xylyl)-, -1-C¹⁴: 743
- ; 1-naphthylcarbamate: 744
- , 1,1,2,2-tetraphenyl-, -1-C¹⁴: 925
- , 2,2'-thiodi-, -S³⁵: 1995
- , 2,2,2-trifluoro-, -1-H₂; and *p*-toluenesulfonate: 1502
- , 2,2,2-trifluoro-, -1-H₂; and *p*-toluenesulfonate: 1501
- , 1,1,2-triphenyl-, -1-C¹⁴: 840, 842, 926
- , 1,1,2-triphenyl-, -C¹⁴₂: 922
- , 1,2,2-triphenyl-, -1-C¹⁴: 419, 841, 923, 926
- ; acetate: 420, 925, 926
- ; *p*-toluenesulfonate: 924, 926
- , 1,2,2-triphenyl-, -C¹⁴₂; and acetate: 924, 926
- , 2,2,2-triphenyl-, -1-C¹⁴: 617, 956
- Ether, allyl 2-(allyl-1-C¹⁴)-6-allylphenyl: 740
- , allyl 2-allyl-1-C¹⁴-phenyl: 739
- , allyl phenyl-2,4,6-H₃: 1322
- , allyl-3-C¹⁴ phenyl: 734, 836
- , allyl-3-C¹⁴ *p*-tolyl: 623, 732
- , allyl 2,6-xylyl-4-H²: 1527
- , allyl-3-C¹⁴ 2,6-xylyl: 734, 735, 736, 737
- , benzyl 2-(5-bromoanisyl): 98
- , *p*-bromophenyl cyclohexyl: 93, 98
- , *p*-bromophenyl isopropyl: 93, 97, 1380
- , *p*-bromophenyl propyl: 1380
- , α -(*o*-bromophenyl)-*p*-tolyl 2-tetrahydropyranyl: 724, 726
- , 3-bromopropyl-3-C¹⁴ phenyl: 733
- , 3-bromopropyl-3-C¹⁴ 2,6-xylyl: 734
- , chloromethyl ethyl: 192
- , 3-chloropropyl-3-C¹⁴ phenyl: 734
- , 3-chloropropyl-3-C¹⁴ *p*-tolyl: 731
- , 3-chloropropyl-3-C¹⁴ 2,6-xylyl: 734
- , α,α -dimethylbenzyl methyl: 1452
- , ethyl-, -O¹⁸: 1883
- , ethyl ethyl-1-C¹⁴: 729
- , 3-iodopropyl-3-C¹⁴ *p*-tolyl: 732
- , 3-iodopropyl-3-C¹⁴ 2,6-xylyl: 734
- , isopropyl phenyl-4-H²: 1380
- , methyl-C¹⁴ triphenylmethyl: 612
- , methyl tris (4-biphenyl) methyl: 616
- , phenyl-4-H² propyl: 1380
- Ethionine (also see Butyric acid, 2-amino-4-(ethylthio)-): 205
- , C¹⁴-: 204
- , -S³⁵: 1956
- , *N*-benzenesulfonyl-: 205
- Ethylamine: 1198
- ; hydrochloride: 1368
- , -1-C¹³: 200, 704
- , -1-C¹⁴: 51, 203, 499, 501
- ; hydrochloride: 499
- ; perchlorate: 915
- , -2-C¹⁴: 51
- ; hydrochloride: 499
- , H²-: 1625
- , -N-H₂; 1368, 1369
- ; hydrochloride-H²: 1368
- , 2-bromo-; hydrobromide: 2000
- , 2-chloro-, -C¹⁴; hydrochloride: 408
- , 2-chloro-*N,N*-diethyl-: 971
- , 2-chloro-*N,N*-dimethyl-: 524
- , 2-chloro-, -1,2-H₂; hydrochloride: 1707

Ethylamine (*Continued*)

- , 2-(3,4-dimethoxyphenyl)-, -1-C¹⁴: 521
- , *N,N*-dimethyl-2-diphenylmethoxy-C¹⁴-; hydrochloride: 524
- , 2,2'-dithiobis[-, -S³⁵: 2000
- , 2-mesityl-2-phenyl-, -1-C¹⁴: 744
- , 2-(*p*-nitrophenyl)-, -1-C¹⁴; and hydrochloride: 519
- , 2-phenyl-, -1-C¹⁴: 919
- , 2,2'-thiobis[-: 1938
- Ethylene: 1228, 1406, 1407, 1408, 1411, 1472, 1680, 1996
- ; platinumous chloride complex: 1407
- , -C¹³: 915
- , -C¹³: 915
- , -C¹⁴: 495
- , -C¹⁴: 867, 913, 915, 966
- , H²:-: 1619
- , -H₂²: 1408
- , -1-H₂²: 1408, 1421, 1422
- , -1,2-H₂²: 1408, 1424, 1479
- ; *cis*:-: 1423, 1424
- ; *trans*:-: 1423, 1424, 1479
- , -H₂²: 1408
- , -H₂²: 1381, 1405, 1407, 1408, 1420, 1421, 1425, 1426, 1473, 1474, 1491
- , -1-H₂²/₁-H₂²/₁: 1412
- , -H₂²: 1706
- , 1,2-bis(phenyl-C¹⁴/₄)-2-phenyl-: 841
- , bromo-, Br⁸⁰: 1168
- , bromo-, -Br⁸²: 1168
- , 1-bromo-, -1-H²: 1478
- , 1-bromo-, -2-H₂²: 1478
- , 1-bromo-, -2-H₂²: 1478
- , 1-bromo-, -1,2-H₂²: 1478
- , 1-bromo-, -H₂²: 1478
- , 2-bromo-1-(*p*-bromophenyl)-1-phenyl-, -1-C¹⁴; *cis*- and *trans*:-: 886
- , 1-(*p*-bromophenyl)-1-phenyl-, -1-C¹⁴: 886
- , bromotriphenyl-: 1231
- , bromotriphenyl-, -Br⁸²: 1159, 1174
- , chloro-, -C¹⁴: 868
- , 1,1-dibromo-, -H₂²: 1478
- , 1,1-dibromo-, -H₂²: 1478
- , 1,2-dibromo-, -Br⁸⁰: 1168
- , 1,2-dichloro-, -H₂²; *cis*- and *trans*:-: 1491
- , 1,1-difluoro-, -H₂²: 1502
- , 1,1-difluoro-, -H₂²: 1502
- , I¹³¹-1,2-diiodo-; *cis*- and *trans*:-: 1253
- , 2-(3,4-dimethoxyphenyl)-1-nitro-, -1-C¹⁴: 521
- , 1,2-diphenyl-, -1-H₂²; *trans*:-: 1460
- , 1,2-diphenyl-, -H₂²; *trans*:-: 1687
- , iodo-, -I¹³¹: 1253
- , iodotriphenyl-, -I¹³¹: 1231
- , 1-(2-naphthyl)-2-phenyl-, -C¹⁴/₂: 843
- , 1-nitro-, -2-C¹⁴: 422
- , 1-phenyl-, -1-H²: 1686
- , phenyl-C¹⁴/₄-diphenyl-: 841
- , poly(-, -H₂²): 1419, 1420
- , tetrachloro-: 873
- , trichloro-: 1491
- , trichloro-, -H²: 1492, 1607
- , triphenyl-: 1159
- , 1,1,2-triphenyl-, -1-C¹⁴: 840, 842
- , triphenyl-, -C¹⁴/₂: 840, 841, 842, 925, 926
- Ethylenediamine: 314, 790
- , -C¹⁴: 497
- ; dihydrochloride: 495, 496
- ; dinitrate: 497
- ; picrate: 497
- , -C¹⁴; and dihydrochloride: 496
- , -C¹⁴/₂: 505
- Ethylene glycol, -C¹⁴: 778, 946
- ; dibenzoate: 779
- , H²:-: 1602
- , triphenyl-: 676
- Ethylene oxide: 43, 214, 259, 260, 304, 734, 877, 1995
- , -C¹⁴: 268, 913, 915, 946
- , -H₂²: 1381, 1382
- , -H₂²: 1706
- Ethylene sulfide, -H₂² (also see Thiirane, -H₂²): 1382
- Ethylenimine (also see Aziridine): 794, 796, 1938
- , -C¹⁴: 408
- , H₂²:-: 1707
- Ethyl mercaptan, 2-(ethylthio)-; sodium derivative: 1924
- 5-Etiobilenic acid, 3 β -hydroxy-; 3-acetate, 16-acid chloride, 17-methyl ester: 1096
- 3 α ,17 β -Etiocolanediol, -11,12-H₂²: 1559
- ; acetate: 1558, 1559
- 11,17-Etiocolanediol, 3 α -hydroxy-, -4,16-H₂²: 1698
- ; acetate: 1679
- 3,11,17-Etiocolanetriol, -4,16-H₂²: 1698
- 3-Etiocolanone, 4-bromo-17 β -hydroxy-, -11,12-H₂²; acetate: 1558
- , 2,4-dibromo-17 β -hydroxy-, -11,12-H₂²; acetate: 1559
- , 17 β -hydroxy-, -11,12-H₂²; acetate: 1558
- 17-Etiocolanone, 3 α -hydroxy-, -5,6,7-H₂²: 1556, 1557
- 4-Etiocolonic acid, 3-oxo-; methyl-C¹⁴ ester: 584

—, 3-oxo-, -3-C¹⁴; and methyl ester: 1061
 —, 3-oxo-, -4-C¹⁴: 1063, 1070
 —; methyl ester: 1063, 1070
 —; sodium salt: 1070
 5-Etiocholenic acid, C¹⁴-3 β -hydroxy-: 1064, 1083
 —; acetate: 1064
 —; methyl ester: 1064
 —, 3-hydroxy-3-methyl-C¹⁴-4-oxa-; methyl ester: 1070
 —, 4-oxa-3-oxo-; methyl ester: 1070, 1071
 4-Etiocholenoyl chloride, 3-oxo-: 1063, 1073, 1074
 —, 3-oxo-, -3-C¹⁴: 1061
 —, 3-oxo-, -4-C¹⁴: 1063, 1070
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 —, C¹⁴-3 β -acetoxy-: 1065
 Equilin: 1162
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 Evans Blue, dibromo-Br₂⁸²: 1164

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 —, - β -C¹⁴: 1038
 —, O-acetyl-, -C¹⁴; ethyl ester: 1029, 1031
 —, α -cyano-C¹⁴-: 1029
 —, O-(tetra-O-acetyl- β -D-glucosyl)-, - α -C¹⁴: 1033
 Ferulnitrile, -C¹⁴: 1029
 —, O-acetyl-: 1030
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 —, O-(tetra-O-acetyl- β -D-glucosyl)-, - α -C¹⁴: 1034
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 —; acetate: 700
 —, 7-hydroxy-2', 4', 6'-trimethyl-, -2-C¹⁴: 701
 —, 7-methoxy-, -2-C¹⁴: 701
 —, 7-methoxy-2', 4', 6'-trimethyl-: 700
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 —, -9-C¹⁴: 386, 387
 —, N-methyl-C¹⁴-; and hydrochloride: 349
 9-Fluorenamine, H²-: 1630
 —, H²-N,N-dimethyl-: 1631
 Fluorene: 109, 110, 389, 631, 2003
 —, -9-C¹⁴: 387, 389
 —, H²-: 1639
 —, -H₁₀²: 1447
 —, 2-acetamido-7-iodo-, -I¹³¹: 1200
 —, N-acetyl-2-amino-, -9-C¹⁴: 388
 —, N-acetyl-2-C¹⁴-2-amino-: 386

—, 2-amino-, -9-C¹⁴: 387
 —, N-benzoyl-2-amino-, -9-C¹⁴: 386
 —, 9-bromo-9-(bromomethyl-C¹⁴)-: 632
 —, 2-iodo-7-nitro-: 1200
 —, H²-9-methoxy-: 1645
 —, 2-methylamino-C¹⁴-: 549
 —, 9-methylene-: 632
 —, 9-methylene-C¹⁴-: 632
 —, 2-nitro-, -9-C¹⁴: 387
 2,7-Fluorenebis(sulfon-*p*-toluidide), -S₂³⁵: 2003
 9-Fluorene-carboxaldehyde, -C¹⁴; α - and β -forms: 631
 3-Fluorene-carboxylic acid, 9-oxo-, -9-C¹⁴: 684
 4-Fluorene-carboxylic acid, 1-methyl-9-oxo-, -C¹⁴₁-9-C¹⁴₁: 683
 —, 3-methyl-9-oxo-, -C¹⁴₁-9-C¹⁴₁: 684
 9-Fluorene-carboxylic acid, -C¹⁴: 109, 850
 —; methyl ester: 850
 —, 2-chloro-9-hydroxy-, -C¹⁴₁-9-C¹⁴₁: 680
 —, 3-chloro-9-hydroxy-, -C¹⁴₁-9-C¹⁴₁: 681
 —, 3-methoxy-, -C¹⁴: 111, 851
 —; methyl ester: 851
 —, 1-methyl-, -C¹⁴: 111, 684
 —; methyl ester: 684
 —, 3-methyl-, -C¹⁴: 111, 682
 —; methyl ester: 682
 2,7-Fluorene-disulfonic acid, -S₂³⁵; disodium salt: 2003
 2,7-Fluorene-disulfonyl chloride, -S₂³⁵: 2003
 9-Fluorene-methanol: 631
 —, - α -C¹⁴: 850
 —, 3-methoxy-, - α -C¹⁴: 851
 —, 1-methyl-, - α -C¹⁴: 684
 —, 3-methyl-, - α -C¹⁴: 682
 9-Fluoreneol: 389
 9-Fluorenone, -9-C¹⁴: 387, 389, 681
 —, 1-chloro-, -9-C¹⁴: 681
 —, 2-chloro-, -9-C¹⁴: 680, 681
 —, 3-chloro-, -9-C¹⁴: 681
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 —, 3-methyl-, -9-C¹⁴; and oxime: 684
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 —, -11-C¹⁴: 309
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 —, *N*-(4,6-diamino-5-pyrimidinyl)-, -C¹⁴; sulfate: 755
 —, *N*-(4,6-diamino-5-pyrimidinyl-1-N¹⁵)-: 1844
 —, *N*-(4,6-diamino-5-pyrimidinyl-1-N¹⁵)thio-: 1845
 —, thio-, -S³⁵: 1940
 Formaldehyde: 32, 483, 550, 638, 639, 910, 918, 944, 1725, 1741, 1775, 1837
 —, -C¹⁴: 33, 169, 170, 172, 207, 247, 249, 308, 314, 364, 383, 422, 460, 484, 506, 532, 607, 608, 612, 613, 614, 616, 632, 639, 672, 719, 733, 736, 738, 741, 948, 952, 957, 980
 —; dimedon derivative: 173, 607, 615, 938
 —; 2,4-dinitrophenylhydrazine: 613, 617
 —, -H₂: 1357, 1359
 —; polymer: 1357
 —, -H₂: 1337, 1338, 1356, 1357, 1359, 1604
 —; polymer: 1356
 —, α -phenylbenzhydryl-(1,1'), (1,2'), (1,3'), (1,4'), (2,2'), (2,3'), (2,4'), (3,3'), (3,4'), (4,4')-C¹⁴₁₀-: 676
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 —, -C¹³: 480
 —, *N*-(6-amino-4-mercapto-5-pyrimidinyl)-, -C¹⁴: 758
 —, *N*-(4,6-diamino-2-methyl-5-pyrimidinyl)-, -C¹⁴; hydrochloride: 755
 —, *N*-(4,6-diamino-5-pyrimidinyl)-, -C¹³; sulfate: 755
 —, *N*-(4,6-diamino-5-pyrimidinyl)-, -C¹⁴; hydrochloride: 754, 755
 —; sulfate: 753
 —, *N*-(4,6-diamino-5-pyrimidinyl-4-C¹⁴)-: 750, 751
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 —; methyl ester: 1669, 1672, 1673
 —, -C¹¹: 33
 —, -C¹³: 480
 —; sodium salt: 32, 33
 —, -C¹⁴: 32, 33, 302, 416, 578, 617, 619, 758, 940, 1294
 —; *S*-benzylthiuronium salt: 625, 631
 —; ethyl ester: 104, 415, 625, 631, 780
 —; methyl ester: 416, 717, 901
 —; potassium salt: 33, 747, 765, 900
 —; sodium salt: 31, 33, 115, 265, 415, 416, 570, 608, 753, 758, 759, 781
 —, -C¹⁴₁₁-H₂¹; ethyl ester: 176, 416, 1294
 —, -H₂: 416, 1259, 1260, 1294
 —; stannous salt: 1359
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 —; 2-chloro-1-(chloromethyl)ethyl ester: 602
 —; ethyl ester: 1719, 1983
 —; methyl ester: 371
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 —, -H₂: 1259, 1260, 1354
 Formimidic acid; ethyl ester: 398, 399, 401
 —; hydrochloride: 1843
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 —, -1-C¹⁴; D-: 996
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 —, -1-H₂: 1378
 —; D-: 1380
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 —, O₁₈-: 1895, 1896
 —, 2,3:4,5-diisopropylidene-, -1-H₂: 1380
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 —; diethyl ester: 1315, 1316
 —; monoethyl ester: 1286
 —, -1-C¹⁴: 125, 146
 —, -2-C¹⁴: 123, 145, 256, 414
 —; dimethyl ester: 124
 —, -2,3-C¹⁴₄: 126, 132, 146, 414
 —, H₂: 1286, 1289
 —; diethyl ester: 1286
 —; dimethyl ester: 1289
 —, O₁₈-: 1894
 —, 2-chloro-, -3-C¹⁴; diethyl ester: 450
 —, 2-hydroxy-, -4-C¹⁴: 351
 Fumaric acid-H₂: 1290
 2-Furaldehyde: 996
 —, 5-(hydroxymethyl)-, -C¹⁴: 994
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 —; D-, cadmium salt: 1013
 —; D-, calcium salt: 1011

- ; D-, γ -lactone: 1011, 1013
 —, -2-C¹⁴; D-: 1013
 —; D-, calcium salt: 1013
 —; D-, γ -lactone: 1013
 Galactononitrile, -1-C¹⁴; D-: 1011
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 —, -1-C¹⁴; α -D-: 1011
 —, -2-C¹⁴; α -D-: 1013
 —, H²:- 1641
 —, O¹⁸:- 1896
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 —; octaacetate: 1024
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 —, 1,2:5,6-di-O-isopropylidene-, -2-C¹⁴; D-: 989
 —, 1,2-O-isopropylidene-, -1-C¹⁴; D-: 986
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 —, -1-C¹⁴: 992, 995
 —; D-: 1006
 —; L-: 996
 —; D-, barium salt: 991, 1007
 —; L-, barium salt: 996
 —; L-, calcium salt: 996
 —; D-, δ -lactone: 991
 —; L-, δ -lactone: 996
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 —, -2-C¹⁴; D-, barium salt: 995, 996
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 —, -6-C¹⁴; D-, potassium salt: 996
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 Glucopyranose; β -D-, pentaacetate: 1026
 —; β -D-1,2,3,4-tetraacetate: 1024
 —, 4-O- β -D-galactopyranosyl-, -1-C¹⁴; D-: 1019
 Glucopyranoside, α -methyl-; L-, tetraacetate: 1027
 —, methyl-, -C¹⁴; D-: 939
 —, α -methyl-C¹⁴-, D-, tetraacetate: 1027
 —, β -methyl-C¹⁴-, D-, tetraacetate: 1026
 Glucosaccharinic acid, -2,2a-C¹⁴_{1/2}; D-: 997
 Glucose; D-, bis(*p*-bromophenyl)osazone: 1153
 —; D-, bis(*p*-bromophenyl)osazone-Br⁸²: 1153
 —; D-, *p*-bromophenylosotriazole-Br⁸²: 1154
 —, C¹⁴-, D-: 994
 —, -1-C¹⁴; D-: 142, 620, 991, 993, 995, 986, 996, 1048
 —; α -L-: 996
 —, -2-C¹⁴: 989
 —; D-: 977, 994, 996
 —; α -D-: 995
 —, -3,4-C¹⁴₂; D-: 143
 —, -6-C¹⁴; D-: 1000, 1002, 1004
 —, -C¹⁴: 1379
 —; D-: 939, 1047, 1048
 —; D-, dimethyl acetal: 939
 —; D-, 1-phosphate: 997
 —, H²:- 1641
 —, 1-H²: 1377
 —; D-: 1380
 —; osazone: 1378
 —, O¹⁸:- 1895
 —, -1-O¹⁸; D-: 1893
 —, acetobromo-: 1037
 —; D- and L-: 1031, 1038
 —, acetobromo-, -C¹⁴: 1024
 —, H²-2-deoxy-: 1641
 —, 6-O- β -D-glucopyranosyl-C¹⁴; D-: 1025
 —; D-, octaacetate: 1024
 —, 4-O- α -D-glucosyl-, -1-C¹⁴; D-: 1023
 —, 1,2-O-isopropylidene-, -6-C¹⁴; D-: 1000, 1004
 —, H²-tetramethyl-: 1642
 Glucoside, α -methyl-; D-: 1893
 —, H²- α -methyl-: 1642
 —, β -methyl-; D-: 1893
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 —; D-, γ -lactone: 1004, 1005
 —; D-, sodium salt: 1004
 —, 1,2-O-isopropylidene-, -6-C¹⁴: 1002, 1004
 —; D-, barium salt: 1000
 —; D-, γ -lactone: 1000, 1002, 1005
 —; D-, sodium salt: 1002
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 —; L-: 210, 1302

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- ; L(+)-: 1634
 —; ammonium-N¹⁵ salt: 1759
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 —, -1,2-C₂¹⁴: 48, 263
 —; L-: 261
 —; hydrochloride: 263
 —; D-, hydrochloride: 263
 —; L-, hydrochloride: 263
 —, -1,2-C₂¹⁴-N¹⁵: 1730
 —, -1,5-C₂¹⁴: 266
 —, -3,4-C₂¹⁴: 267
 —, -2-H²; L-: 1299, 1300
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 —, -4-H₃²; L-: 1302
 —, -2,3-H₃²: 1297, 1302, 1316
 —, -2,3-H₃²-N¹⁵: 1793
 —, -N¹⁵: 1729, 1730
 —; L-: 1730
 —; barium salt: 1729
 —, N-acetyl-, -N¹⁵: 1730
 —, N-acetyl-H₃²-; L-: 1291
 —, N-*p*-aminobenzoyl-, L-: 308, 312
 —, N-*p*-[(2-amino-4-hydroxy-6-pteridylmethyl-C¹⁴)amino]-benzoyl-; L-: 308
 —, N-*p*-[(2-amino-4-hydroxy-6-pteridylmethyl-2-C¹⁴)amino]benzoyl-; L-: 311
 —; L-, diethyl ester, hydrobromide: 311
 —, N-*p*-[N-(2-amino-4-hydroxy-6-pteridylmethyl-2-C¹⁴)-*p*-toluenesulfonamido]benzoyl-; L-, diethyl ester: 310
 —, N-carbamoyl-, -2-H²; L-: 1299
 —, N-carbamoyl-, -3-H₃²; L-: 1301
 —, N-carbamoyl-, -4-H₃²; L-: 1302
 —, N-carbamoyl-C¹⁴-; L-: 306
 —, C₂¹⁴-H₃²-N¹⁵-N-(*o*-carboxyethyl)-: 1794
 —, N-pteroyl-2-C¹⁴-; L-: 311, 312
 —, N-[N-*p*-toluenesulfonyl-N-(2-oxo-3,3-diethoxypropyl)-*p*-aminobenzoyl]-; L-, diethyl ester: 311
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 —, N²-benzyloxycarbonyl-, -N-N¹⁵; benzyl ester: 1759
 —, N-(carboxymethyl-C₂¹⁴)-: 297
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 —, 2-amino-, -5-N¹⁵: 1759
 Glutaric acid, -1,5-C₂¹⁴; and monopiperazine salt: 452
 —, H²-: 1596
 —, 2-acetamido-, -N¹⁵: 1730

- , 2-amino-, -1,2-C₂¹⁴-N¹⁵: 1730
 —, 2-amino-, -2,3-H₃²-N¹⁵: 1793
 —, 2-amino-, -N¹⁵: 1729, 1730
 —, 2-carboxy-2,3-dimethyl-, -5-C¹⁴; triethyl ester: 136
 —, 2-carboxy-3,4-dimethyl-, -1-C¹⁴: 136
 —; triethyl ester: 135
 —, 2-carboxy-3,4-dimethyl-, -1-O₁¹⁸: 1867
 —, 2-carboxy-3,4-dimethyl-, -O₄¹⁸; and trimethyl ester: 1869
 —, 2-carboxy-3-methyl-, -5-C¹⁴; and triethyl ester: 136
 —, 2-carboxy-4-methyl-3-phenyl-, -O₄¹⁸: 1870
 —; trimethyl ester: 1869
 —, 3-carboxy-2-oxo-, -1,2-C₂¹⁴; trimethyl ester; potassium derivative: 262
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 —, 2-ethoxycarbonyl-2,3-dimethyl-, diethyl ester: 1867
 —, 2-ethoxycarbonyl-O¹⁸-3,4-dimethyl-, diethyl ester: 1866
 —, 3-hydroxy-3-methyl-, -3-C¹⁴: 133
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 —; ethyl ester: 932
 —, O,O'-diacetyl-, -1-C¹⁴; ethyl ester: 933
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 —, -1-C¹⁴: 932, 938
 —; tribenzoate: 937
 —, -1,3-C₂¹⁴: 936
 —; 10,12-linoleate: 437
 —; 10,12-octadecadienoate: 437
 —, -C₃¹⁴; and tribenzoate: 940
 —, H²-: 1602
 —, -1-H₁²: 1352
 —, 1,2-isopropylidene-: 1908
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 —, N-(N-benzyloxycarbonylglycyl-N¹⁵)-: 1757, 1758

- , *N*-glycyl-, -2-N¹⁵; acetate: 1758
- , *N*-glycyl-N¹⁵-; acetate: 1758
- Glycine: 1180, 1717, 1762
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- ; ethyl ester, hydrochloride: 163
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- ; ethyl ester, hydrochloride: 244
- , -1-C¹³-2-C¹⁴: 163
- ; ethyl ester, hydrochloride: 246
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- , -1-C¹⁴: 159, 163, 289
- , -2-C¹⁴: 160, 235, 246, 292, 403, 718
- ; ethyl ester, hydrochloride: 246, 465, 718
- , -C¹⁴₁: 292, 297
- , -C¹⁴₂: 948
- , H²-: 1633
- , -2-H²-N¹⁵: 1732
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- , *N*-acetyl-: 222, 348
- , *N*-acetyl-, -1-C¹⁴: 163
- , *N*-acetyl-H³-: 1291
- , *N*-amidino-, -N¹⁵: 1753
- , *N*-amidino-*N*-methyl-C¹⁴-: 693
- , *N*-amidino-*N*-methyl-H³-: 1293
- , *N*-benzoyl-, -1-C¹⁴: 289
- , *N*-benzyloxycarbonyl-; benzenethio ester: 1758
- , *N*-benzyloxycarbonyl-, -N¹⁵: 1717
- , *N*-(*N*-benzyloxycarbonylglycyl)-, -N¹⁵: 1757, 1758
- ; ethyl ester: 1757
- , *N*-(*N*-benzyloxycarbonylglycyl-N¹⁵)-; and ethyl ester: 1757
- , *N*-(*N*-benzyloxycarbonyltryptophyl)-, -N¹⁵: 1758
- , *N*-carbamoyl-, -N¹⁵: 1756
- , *N*-carbamoyl-*N*-methyl-, -N¹⁵: 1756
- , *N*-(*N*-carbobenzyloxy-γ-L-glutamyl)-, -C¹⁴: 297
- , *N*-chloroformyl-, -1-C¹³: 715
- , *N,N*-dimethyl-, -1-C¹⁴; ethyl ester: 964
- , *N*-γ-L-glutamyl-, -C¹⁴: 297
- , *N*-glycyl-, -N¹⁵: 1757
- , *N*-glycyl-N¹⁵-: 1757
- , *N*-methyl-, -N¹⁵; hydrochloride: 1801
- , *N*-methyl-C¹⁴-: 166, 967
- ; hydrochloride: 692, 969
- ; *p*-toluenesulfonic acid salt: 969
- , *N*-methyl-H³-; hydrochloride: 1292
- , *N*-methyl-*N*-*p*-toluenesulfonyl-, -N¹⁵: 1800
- , *N*-methyl-C¹⁴-*N*-*p*-toluenesulfonyl-: 692, 969
- , H²-2-phenyl-: 1635
- , C¹⁴-poly-: 715
- , *N*-*p*-toluenesulfonyl-: 692, 969, 1292
- , *N*-*p*-toluenesulfonyl-, -N¹⁵: 1800
- Glycine anhydride, *N*-carboxy-: 1718
- Glycocyamine, N¹⁵-: 1753
- Glycolaldehyde: 144, 933
- ; cyanohydrin-C¹⁴: 933
- , -C¹⁴₂; 2,4-dinitrophenylhydrazones: 940
- , α-*D*-hydroxymethyl-C¹⁴-α'-*L*-methoxydi-, -C¹⁴: 940
- Glycol-C¹³₃ aldehyde, D'*L*'-methoxy-*D*-hydroxymethyl-: 940
- Glycolic acid: 454
- , -1-C¹⁴: 137, 636
- ; acetate: 636
- ; calcium salt: 137
- , -2-C¹⁴; and calcium salt: 137
- , -C¹⁴₂: 994
- , H²-: 1595
- , bis (chloromethyl)-: 156
- , bis (cyano-C¹⁴-methyl)-; potassium salt: 156
- , (*m*-chlorophenyl)phenyl-, -C¹⁴₂: 151
- , (*o*-chlorophenyl-, -C¹⁴₂: 151
- , (*p*-chlorophenyl-, -C¹⁴₂: 151
- , diphenyl-, -C¹⁴₂: 152
- , (*p*-methoxyphenyl)phenyl-, -C¹⁴₂: 150, 151
- , phenyl-, -1-C¹⁴: 146
- , phenyl-2-thienyl-, -C¹⁴₂: 153
- , phenyl-3-thienyl-, -C¹⁴₂: 155
- , phenyl-*p*-tolyl-, -C¹⁴₂: 151
- Glycolic anhydride, bis (-, -1-C¹⁴): 138
- Glycolonitrile, -1-C¹⁴: 944
- Glycoluric acid, -1-C¹³: 245
- Glycyl chloride, *N*-benzyloxycarbonyl-: 1757
- , *N*-benzyloxycarbonyl-, -N¹⁵: 1717, 1757, 1758
- Glyoxal, -C¹⁴; and 2,4-dinitrophenylsone: 814
- , -H³: 1335, 1357
- ; bis (cyanohydrin): 1335
- ; bis (phenylhydrazones): 1334
- ; bisulfite addition product: 1335
- , 1-(*m*-chlorophenyl)-2-phenyl-, -2-C¹⁴: 678

Glyoxal (*Continued*)

- , 1-(*o*-chlorophenyl)-2-phenyl-, -2-C¹⁴: 678
- , 1-(*p*-chlorophenyl)-2-phenyl-, -2-C¹⁴: 678
- , 2-(*m*-chlorophenyl)-1-phenyl-, -1-C¹⁴: 151
- , 2-(*o*-chlorophenyl)-1-phenyl-, -1-C¹⁴: 151
- , 2-(*p*-chlorophenyl)-1-phenyl-, -1-C¹⁴: 151
- , 1-(*p*-methoxyphenyl)-2-phenyl-, -C¹⁴: 149, 150, 678
- , 2-(*p*-methoxyphenyl)-1-phenyl-, -1-C¹⁴: 151
- , methyl-, -2-C¹⁴; disemicarbazone: 641
- , 2-phenyl-, -2-C¹³; hydrate: 149
- , 2-phenyl-, -2-C¹⁴; hydrate: 148
- ; phenylosazone: 148
- , 1-phenyl-2-(2-thienyl)-, -1-C¹⁴: 153, 154
- , 1-phenyl-2-(3-thienyl)-, -1-C¹⁴: 155
- , 1-*p*-tolyl-2-phenyl-, -2-C¹⁴: 678
- , 2-*p*-tolyl-1-phenyl-, -1-C¹⁴: 151
- Glyoxime, H²-dimethyl: 1656
- Glyoxylic acid; phenylhydrazone: 463
- , -2-C¹⁴; 2,4-dinitrophenylhydrazone: 157
- ; sodium salt: 157
- , -C₂¹⁴: 462, 464
- Gramine: 251
- , C₁¹⁴—: 247, 335, 337
- , N₁¹⁵—: 1775
- Guanidine, -C¹³; nitrate: 592, 757
- , -C¹⁴; hydrochloride: 591, 592, 756, 761
- ; nitrate: 591, 592
- ; picrate: 591, 592
- , -N₃¹⁵: 1849
- , cyano: 1846
- , cyano-, -C₂¹⁴: 589, 592
- , (cyanoacetyl)-, -N₃¹⁵: 1849
- Guanidinium bromide, -N₃¹⁵: 1848
- Guanidinium nitrate, -N₃¹⁵: 1846
- , -1,2-N₁¹⁵₂: 1808, 1809
- Guanine: 1677
- , -4-C¹³: 764
- , -2-C¹⁴; and sulfate: 761
- , -4-C¹⁴: 764
- ; hydrochloride: 763
- , -8-C¹⁴: 760, 765, 766
- ; hydrochloride: 765
- ; sulfate: 766
- , -8-H³: 1676
- , -1,2,3-N₃¹⁵; and sulfate: 1850
- , 9-β-D-ribofuranosyl-, -2-C¹⁴: 1045
- Guanosine, -2-C¹⁴: 1045

- Gulonic acid, 2,3: 4,6-di-*O*-isopropylidene-2-oxo-, -C₆¹⁴; L-: 1127
- , 2,3-dioxo-, -1-C¹⁴; L-: 1125
- ; L-, calcium salt: 1125
- , 2-oxo-, -C₆¹⁴; L-: 1127
- ; L-, methyl ester: 1127

II

- Hematoporphyrin, C₂¹⁴; dimethyl ester: 471
- Hendecanoic acid, -C¹⁴: 96
- Heptadecanoyl chloride, 11-carbomethoxy-: 65
- 8,11-Heptadecadiene, 1-bromo-: 435, 436
- 8,11-Heptadecadien-1-ol; and *p*-toluenesulfonate: 436
- Heptadecane, 1-bromo-: 96
- , 1,8,9,11,12-pentabromo-: 436
- , 1,8,9-tribromo-; *threo*-: 68
- 8,9-Heptadecanediol, 1-bromo-; *erythro*-: 67, 69
- ; *erythro*-, diacetate: 69
- Heptadecan-1-ol, 8,9,11,12-tetrabromo-: 436
- 8-Heptadecene, 1-bromo-; *cis*- and *trans*-: 68
- 1,6-Heptadien-4-ol, 4-methyl-, -4-C¹⁴: 134
- 2,5-Heptadien-4-one, 3-isopropyl-2-methyl-: 1284
- Heptanal: 1999
- ; sodium bisulfite-S³⁵ addition product: 1999
- Heptanamide, -1,7-C₁₄¹⁴: 503
- Heptane, H²-: 1616
- , 1-bromo-: 43, 96
- , 1-bromo-, -1-C¹⁴: 37, 882
- , 1-bromo-, -1-H₂³: 1429
- , 3-bromo-: 91, 96
- , H²-3-methyl-: 1617
- Heptanedioic acid, -1-C¹⁴; and calcium salt: 659
- , -1,7-C₂¹⁴: 823
- , 2-carboxy-, -2-C¹⁴: 281
- 1-Heptanesulfonic acid, 1-hydroxy-, -S³⁵; sodium salt: 1999
- Heptanoic acid; ethyl ester: 1429
- , -1-C¹⁴: 37, 96, 882
- , -7-C¹⁴: 59
- , C¹⁴-6-amino-; hydrochloride: 1109
- , 2,2-dimethyl-6-oxo-, -7-C¹⁴: 355
- , 5-oxo-: 60
- , 2,4,6-trioxo-, -3,5-H₄²; ethyl ester: 1388
- 1-Heptanol, -1-C¹⁴: 37, 882
- , -1-H₂³: 1429

- , 1-methyl-; chlorosulfite ester: 1345
- 4-Heptanol, H^2 -2,2,6,6-tetramethyl-: 1602
- 2-Heptanone, -1- C^{14} : 503, 646
- , 6-hydroxy-6-methyl-, -1- C^{14} : 355
- 3-Heptanone, 6-dimethylamino-4,4-diphenyl-, -1- C^{14} ; hydrobromide: 653
- , 6-dimethylamino-4,4-diphenyl-, -2- C^{14} ; hydrobromide: 653
- 1-Heptene, 1-bromo-: 1173
- 6-Heptenoic acid, 3-oxo-; ethyl ester: 467
- 2-Hepten-4-one, 5-oxa-, -4- O^{18} : 1866
- 5-Hepten-2-one, 6-methyl-: 650
- , 6-methyl-, -1- C^{14} : 355
- Heptylamine, H^2 -: 1628
- Heptyl bromide, -1- C^{14} (also see Heptane, 1-bromo): 882
- Heteroauxin, C_1^{14} -: 335
- Hexachlorophene, C_1^{14} -: 957
- Hexadecane, -1,2- H_2^2 : 1417
- Hexadecanoic acid, -1- C^{13} ; potassium salt: 434
- ; silver salt: 434
- , -1- C^{14} : 432, 673
- ; 3-chloro-2-oxopropyl-3- C^{14} ester: 455
- ; 3-diazo-2-oxopropyl-3- C^{14} ester: 455
- ; 2,3-dihydroxypropyl-3- C^{14} ester: 455
- ; glyceryl ester: 433, 434
- ; 3-hydroxy-2-oxopropyl-3- C^{14} ester: 455
- ; methyl ester: 433
- ; sodium salt: 434
- , -2- C^{14} : 37
- , -3- C^{14} : 53
- , -5- C^{14} : 65
- , -6- C^{14} : 64
- ; glyceryl ester: 434
- , -11- C^{14} : 65
- ; glyceryl ester: 434
- , -13- C^{14} : 65
- , -15- C^{14} : 65
- , -2- H_2^2 : 1311
- , 2-bromo-, -2- H^2 : 1311
- , 15,15-dimethyl-: 72
- , 5-oxo-, -6- C^{14} ; and methyl ester: 64
- , 10-oxo-, -11- C^{14} ; and ethyl ester: 65
- , 13-oxo-, -15- C^{14} : 65
- 1-Hexadecanol: 2005
- Hexadecanophenone, -1- C^{14} : 673
- , 2-hydroxyimino-, -1- C^{14} : 674
- Hexadecanoyl chloride, -1- C^{14} : 433, 454, 673
- 2,4-Hexadiene, 2,5-dimethyl-: 465
- 2,4-Hexadienoic acid; ethyl ester: 1267
- 1,5-Hexadien-3-one, 2-methyl-: 1285
- Hexamethylenimine, C^{14} -2-methyl-7-oxo-: 1109
- , C^{14} -3-methyl-2-oxo-: 1109
- , 2-oxo-, -3,4,5- H_2^2 : 1326
- Hexane, H^2 -: 1614
- , 1-bromo-: 96
- , 1-bromo-, -1- C^{13} : 53, 882
- , 1-bromo-, -1- C^{14} : 53, 65, 882
- , H^2 -2,3-dimethyl-: 1617
- , H^2 -3,3-dimethyl-: 1617
- , H^2 -2-methyl-: 1616
- , H^2 -3-methyl-: 1616
- 1,6-Hexanediamine, C^{14} -: 538
- Hexanedinitrile, -1,6- C_2^{14} : 828
- Hexanedioic acid, -1- C^{13} ; barium salt: 658
- , C^{14} -: 538
- , -1- C^{14} : 661
- , -1,6- C_2^{14} : 85, 827, 828
- , -1,2- C_1^{14} ; and barium salt: 658
- , 2-amino-, -6- C^{14} : 270, 271
- ; hydrochloride: 270
- ; L-: 270
- , 2-benzamido-: 271
- , N-carbobenzyloxy-2-amino-, -6- C^{14} : 270
- ; L-, anilide: 270
- , 2-carboxy-3-oxo-, -2- C^{14} ; triethyl ester: 352
- , 3-oxo-: 1751
- , 3-oxo-, -2- C^{14} : 352
- Hexaneitrile: 646
- , 2,6-dihydroxy-, -1- C^{14} : 277
- Hexanoic acid, -1- C^{13} : 43
- ; ethyl ester: 882
- , -1- C^{14} : 96, 882
- , -2- H_2^2 : 1268
- , -2,3,4,5- H_2^2 ; and sodium salt: 1267
- , 2-acetamido-6-amino-, -6- C^{14} ; ethyl ester: 282
- , 2-acetamido-2-cyano-6-phthalimido-, -2- C^{14} ; ethyl ester: 280
- , 2-acetamido-4-thia-5-oxo-, - S^{35} : 1966
- , 2-amino-, - N^{15} : 1729
- , 6-amino-, -1,2,6- C_1^{14} : 540
- , 2-amino-6-benzamido-, -2- N^{15} ; L-: 1790
- , 6-benzamido-, -3,4,5- H_2^2 : 1326
- , 6-benzamido-2-bromo-; D-: 1790, 1791

Hexanoic acid (*Continued*)

- , 6-benzamido-2-bromo-, -3,4,5- H_3^2 : 1327
 —; ethyl ester: 1327, 1788
 —, 6-benzamido-2-chloro-; ethyl ester: 1785, 1787
 —, 6-benzamido-2-phthalimido-, -3,4,5- H_3^2 -2- N^{15} ; ethyl ester: 1788
 —, 6-benzamido-2-phthalimido-, -2- N^{15} ; ethyl ester: 1785
 —, 2,6-diamino-, -1- C^{14} ; dihydrochloride: 275
 —, 2,6-diamino-, -2- H^2 -2- N^{15} : 1786
 —, 2,6-diamino-, -3,4,5- H^2 -2- N^{15} : 1788
 —, 2,6-diamino-, -2- N^{15} ; dihydrochloride: 1786
 —; L-: 1790
 —, 2,6-diamino-, -6- N^{15} ; dihydrochloride: 1792
 —, 2-ethyl-, -1- C^{14} : 96, 431
 —, 2-oxo-: 1729, 1730
 —, 3-oxo-; ethyl ester: 710, 1975
 Hexanoin, 1,3-di-, -1,3- C_2^{14} : 935
 1-Hexanol, -1- C^{13} : 882
 —, -1- C^{14} : 882
 —, 2-ethyl-, -1- C^{14} : 431
 —; decanedioate: 432
 2-Hexanone, H^2 -: 1605
 3-Hexanone, 2,2-dimethyl-4-oxa-, -3- O^{18} : 1880
 Hexanoyl chloride: 935
 3-Hexenoic acid, 4-methyl-; lithium salt: 355
 Hexestrol, H_2^2 -: 1529
 —, H_3^2 -: 1692
 —, H_4^2 -: 1690, 1691
 —; *meso*-: 1691, 1692
 Hexylamine, -6- C^{14} ; hydrochloride: 503
 Hexyl bromide, -1- C^{13} (also see Hexane, 1-bromo-): 882
 —, 1- C^{14} : 882
 Hippuric acid: 222
 —; ethyl ester: 1294
 —, - C^{13} : 292
 —, - C^{13} - N^{15} ; ethyl ester: 175, 1742
 —, - α - C^{13} : 220, 292
 —, - C^{14} : 233, 289, 292
 —, - C^{14} - α - C^{14} : 220, 292
 —, - α - C^{14} : 178, 190, 292
 —, - N^{15} : 1746, 1748
 —; ethyl ester: 1745, 1746
 —; sodium salt: 1748
 —, α -formyl-, - C^{13} - N^{15} ; ethyl ester: 175, 1742
 —, α -formyl-, - N^{15} ; ethyl ester: 1745, 1746
 —, α -formyl- C_1^{14} - $H_1^{1/2}$ -; ethyl ester: 176, 1294

Histamine: 530

—, C_1^{14} -; dihydrochloride: 527, 529

Histidine: 530, 1207

- ; methyl ester, dihydrochloride: 1983
 —, C_1^{14} -: 289, 291
 —; dihydrochloride: 239
 —, - α - C^{14} ; and dimercury derivative: 293
 —, -2- C^{14} ; L-: 295, 296, 529
 —; L-, diflavianate: 295
 —; L-, hydrochloride: 296
 —, -3(or 1)- N^{15} ; L-: 1773
 —; L-, diflavianate: 1773
 —; L-, monohydrochloride: 1773
 —, C_1^{14} - N -benzoyl-: 289
 —, 2-(carbethoxythio)-, - S^{35} ; dihydrochloride: 1983
 —, 2,5-diiodo-, - I_2^{31} : 1207
 —, 2-iodo-, - I^{31} : 1207
 —, 2-mercapto-, -2- C^{14} ; L-: 294, 296
 —, 2-mercapto-, -3(or 1)- N^{15} ; L-: 1773
 —; L-, dihydrochloride: 1774
 —, 2-mercapto-, - S^{35} : 1982
 Homocitrulline, C_1^{14} -; L-, hydrochloride: 210
 Homocysteine (also see Butyric acid, 2-amino-4-mercapto-): 1302
 —; sodium salt: 204
 —, H_2^2 -: 1306
 —, - S^{35} : 1971, 1972
 —; sodium salt: 1973
 —, N -acetyl- S -benzyl-: 205
 —, N -acetyl- S -benzyl-, - S^{35} : 1962
 —; D-: 1959
 —; L-, anilide: 1962
 —, S -benzyl-: 205, 1303
 —; D-: 199
 —; L-: 198, 205
 —, S -benzyl-, -3,4- C_{13}^{13} : 203
 —, S -benzyl-, - S^{35} : 1957, 1958, 1959
 —; D-: 1958
 —; L-: 1958
 —, C_2^{13} - S -benzyl-, - S^{34} : 1959
 —, H_2^2 - S -benzyl-: 1305
 —, S -ethyl-1- C^{14} -: 204
 —; L-: 205
 Homocystine: 204
 —; L-: 199
 —, H_2^2 -: 1306
 —, - S_2^{35} : 1971, 1972
 16-Homo-5-etiobilenamide, 3 β -hydroxy-, -16- C^{13} ; 3-acetate, 17-methyl ester: 1095
 16-Homo-5-etiobilenic acid, 3 β -hydroxy-, -16- C^{13} : 1096
 16-Homo-5-etiobilenic anhydride, 3 β -hydroxy-, -16- C^{13} ; 3-acetate: 1096

- Homolanthionine, -S³⁵: 1965
- Homomarrarianolic acid, C¹⁴; dimethyl ester, methyl ether: 1092
- Hydantoic acid, -1-C¹³; ethyl ester: 245
- , -3-N¹⁵: 1756
- , 5-(*p*-chlorophenyl)-, -C¹³: 1180
- , 3-methyl-, -3-N¹⁵: 1756
- , 5-phenyl-, -1-C¹³: 715
- Hydantoin: 222, 227, 237
- , -2-C¹³: 396
- , -4-C¹³: 245
- , -2-C¹⁴: 775
- , -4-C¹⁴: 775
- , -N₂¹⁵: 1797
- , acetylthio-: 222
- , 5-(4-aminobutyl)-, -4-C¹⁴: 277
- , 5-(4-benzamidobutyl)-, -4-C¹⁴: 276
- , 5-(4-bromobutyl)-, -4-C¹⁴: 277
- , 5-bromo-5-carboxymethylene-, -1-N¹⁵: 1714
- , 5-carboxyimino-N_{1/1}¹⁵-, -1,3-N_{2/1}¹⁵; potassium salt: 1798
- , 5-carboxymethylene-, -1-N¹⁵: 1714
- , 5-(carboxy-C¹⁴-methylene)-: 357, 359, 362
- , 5-(3,4-dimethoxybenzyl)-, -4-C¹⁴: 236
- , 5,5-diphenyl-, -4-C¹⁴: 694
- , 5-ethyl-3-methyl-5-phenyl-, -4-C¹⁴: 194
- , 5-ethyl-5-phenyl-, -4-C¹⁴: 193
- , 5-ethyl-5-phenyl-, -1-N¹⁵: 1798
- , 5-(4-hydroxybutyl)-, -4-C¹⁴: 277
- , 5-(3-indolylmethyl)-, -4-C¹³: 245
- , 5-(3-indolylmethylene)-, -4-C¹³: 245
- , 5-(*p*-methoxybenzyl)-, -4-C¹⁴: 230, 232
- , 5-(*p*-methoxybenzylidene-C¹⁴)-: 227
- , 5-(1-methylethyl-2-C¹³)-: 193
- , 5-[2-(methylthio)ethyl]-, -S³⁵: 1953, 1955
- , 5-ureido-, -2-C¹⁴: 775
- , 5-ureido-, -4-C¹⁴: 775
- , 5-ureido-C¹⁴-: 773, 775
- , 5-(ureido-1,3-N_{2/1}¹⁵)-, -1,3-N_{2/1}¹⁵: 1797, 1799, 1804
- , 5-veratryl-C¹⁴-: 238
- , 5-veratrylidene-C¹⁴-: 237
- 5-Hydantoinacetic acid, -C¹⁴: 359
- , -1-C¹⁴: 362
- , -2-C¹⁴: 357, 359
- , D- and L-: 359
- , -1-N¹⁵: 1714
- Hydracrylic acid, -1-C¹¹: 43
- , -2-C¹³-1-C¹⁴: 704
- , -1-C¹⁴: 115
- , O₁¹⁸; β -lactone: 1874
- , 3,3-diphenyl-, -3-C¹⁴; ethyl ester: 838
- ; hydrazide: 839
- , 3-ethylidene-2-hexyl-, -3-C¹⁴; β -lactone: 482
- , 3-heptylidene-2-methyl-, -1-C¹⁴; β -lactone: 482
- , 2-phenyl-, -2-C¹⁴: 550
- ; 3-(8-methyl-8-azabicyclo[3.2.1]octyl) ester: 551
- Hydracrylonitrile, -1-C¹⁴: 168, 257, 259, 260, 731, 734
- , -2,3-C₂¹⁴: 268
- Hydratropic acid, H²-: 1598
- ; ethyl ester: 1644
- Hydrazine; hydrate: 1201, 1795
- , 1-(4-acetamido-4,4-diethoxycarbonylbutylidene)-2-phenyl-H₂²-: 1308
- , 1-benzenesulfonyl-2-benzoyl-C¹⁴-: 628
- , 1,2-bis(1-phthalazinyl-1-C¹⁴)-: 793
- , 4-bromophenyl-, -Br⁸²: 1153
- , (*N*-carbobenzyloxy- γ -L-glutamyl)-: 297
- , 2,4-dinitrophenyl-: 940, 1558, 1574
- , 1,1-diphenyl-, -2-N¹⁵; and hydrochloride: 1830
- , 1-formyl-C¹³-2-phenyl-: 480
- , 1-isopropylidene-2-phenyl-, -1-N¹⁵: 1832
- , 1-(α -methylbenzylidene)-2-phenyl-, -2-N¹⁵: 1811
- , *p*-nitrophenyl-: 48
- , 1-(*p*-nitrophenyl)-, -2-N¹⁵: 1825
- ; hydrochloride: 1826
- , phenyl-: 480, 993, 1308, 1378, 1855, 1856
- , 1-phenyl-, -1-N¹⁵: 1832
- ; hydrochloride: 1811
- , 1-phenyl-, -2-N¹⁵: 1715, 1829, 1832
- ; hydrochloride: 1812, 1829
- , phenyl-H₂²; and hydrochloride: 1308
- , triphenyl-, -N_{1/2}¹⁵: 1833
- Hydrazobenzene, -4,4'-H₂²: 1592
- , -N₁¹⁵: 1723, 1831, 1833
- , 2-ethoxy-2'-methyl-C¹⁴-: 545
- , 2-methyl-C¹⁴-: 545
- Hydrazoic acid: 799, 1960
- , 1-N¹⁵; potassium salt: 1795
- ; sodium salt: 1855
- 2-Hydrindone-1-carboxylic acid, -C¹⁴-2-C¹⁴; ethyl ester: 458
- Hydriodic acid, I¹³¹; calcium salt: 1238, 1245
- ; potassium salt: 1195, 1197, 1224, 1226, 1231, 1240, 1250
- ; sodium salt: 1195, 1199, 1200, 1201, 1203, 1204, 1205, 1208, 1209, 1216, 1219, 1228, 1229,

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 1232, 1235, 1239, 1242, 1243,
 1244, 1246
 Hydriodic acid- H_2 : 1277
 Hydrobenzoin, $-\alpha, \alpha'-H_2$: 1365, 1367
 —, 3,4,3',4'-bis(methylenedioxy)-,
 $\alpha, \alpha'-H_2$: 1367
 —, 4,4'-dichloro-, $-\alpha, \alpha'-H_2$: 1367
 Hydrobromic acid, $-Br^{80}$; lithium salt:
 1166
 —, $-Br^{82}$: 1151, 1162
 —; potassium salt: 1154, 1162,
 1166
 —; sodium salt: 1162
 Hydrochloric acid, $-Cl^{36}$: 1187, 1189
 —; lithium salt: 1185
 —; sodium salt: 1193
 —, $-Cl^{38}$; potassium salt: 1186, 1187
 Hydrochloric acid- H_2 : 1300, 1302,
 1342, 1384, 1418, 1517
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 Hydrocinnamic acid, $-\alpha-C^{14}$: 885
 —, $-\beta-C^{14}$: 76
 —, $-\alpha-\beta-C^{14}_2$: 885
 —, H_2 —: 1277, 1598
 —; strychnine salt: 1278
 —, $-\alpha-H_2$: 1276
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 —, 4-benzylidene-1,2,3,4- C^{14}_4 -2-phenyl-, -4- C^{13} : 220
 —, 2-benzyl-4-methoxymethylene-: 1986
 —, 4-[3,5-diiodo-4-(*p*-methoxyphenoxy)benzylidene]-2-phenyl-, -5- C^{14} : 233
 —, 4-(*p*-hydroxybenzylidene)-2-methyl-, - N^{15} ; acetate: 1762

—, 4-(1-hydroxyethylidene)-2-phenyl-, -4- C^{14} : 178
 —, 4-isopropylidene-2-methyl-: 1985
 —, 4-isopropylidene-2-phenyl-, -4- C^{14} : 190
 Oxonic acid, -2- C^{14} ; potassium salt: 773
 Oxygen, - O^{18} : 1877

P

Palmitic acid(also see Hexadecanoic acid): 71, 1311
 —, $-C^{14}$: 432, 454
 —; 2,3-dihydroxypropyl-3- C^{14} ester: 455
 —; glyceryl ester: 433
 —, -6- C^{14} : 64
 —, H^2 :- 1270
 —, -2- H^2 :- 1311
 —, H^3 :- 1668
 —, 2-bromo-, -2- H^2 :- 1311
 Palmitin, 1,3-di-: 434
 —, C^{14}_2 - α -mono-: 455
 —, C^{14}_3 -tri-: 433
 Palmitophenone, C^{14} :- 673
 Palmitoyl chloride, $-C^{14}$: 433
 Papain: 212, 224, 285, 1786, 1962
 Paraformaldehyde: 717, 1837
 —, $-C^{14}$: 612, 672, 934
 Paraldehyde, - H^2_2 :- 1364
 Parathion- P^{32} : 1925, 1926
 —, - P^{32} - S^{35} : 1988
 —, - S^{35} : 1990
 Pelentan, C^{14}_2 :- 463
 Penicillamine, - S^{35} ; hydrochloride: 1986
 —, *N*-acetyl-, - S^{35} : 1985
 Penicillic acid, benzyl-, - S^{35} : 1987
 Penicillin, benzyl-; sodium salt: 1588
 —, benzyl-, - S^{35} ; sodium salt: 1987
 —; triethylammonium salt: 1986
 —, dethiobenzyl-: 1588
 —, H^2 -dethiobenzyl-: 1588
 Penicillin-G: 1588
 Pentadecane, 1-bromo-, -1- C^{14} : 37
 Pentadecanenitrile: 674
 Pentadecanoic acid: 674
 —, -1- C^{14} : 37, 96
 —, -5- C^{14} : 65
 —, 4-oxo-, -5- C^{14} ; methyl ester: 65
 1-Pentadecanol: 433
 —, -1- C^{14} : 37
 Pentaerythritol, H^2 :- 1641
 Pentamethylene bromide(also see Pentane, 1,5-dibromo-): 1816
 Pentane, -1- C^{14} : 909
 —, -3- C^{14} : 909
 —, 1-bromo-: 43

Pentane (*Continued*)

- , 1-bromo-, -1-C¹⁴: 870
- , 2-bromo-, -1,3-H₃²: 1485
- , 3-(bromomethyl)-, -3-H²: 1487
- , 1-bromo-4-methyl-: 1416
- , 2-bromo-4-methyl-: 1415, 1416
- , 1-chloro-, -Cl³⁶: 1191
- , 2-chloro-, -1,3-H₃²: 1345
- , 1-chloro-5-isopropylamino-; hydrochloride: 1814, 1820
- , 2-chloro-2-methyl-: 1415, 1416
- , 1,5-dibromo-: 102, 823, 1816
- , 1,5-dibromo-3-methyl-: 104
- , H²-2,3-dimethyl-: 1615
- , H²-2,4-dimethyl-: 1615
- , H²-3-ethyl-: 1616
- , 1-iodo-, -I¹²⁸: 1255
- , 1-iodo-, -I¹³¹: 1255
- , 2-iodo-, -I¹³¹: 1255
- , 2-methyl-, -2-H²: 1415
- , 2-methyl-, -4-H₃²: 1415
- , 2-methyl-, -5-H₃²: 1416
- , H²-2-methyl-: 1615
- , H²-3-methyl-: 1615
- 1,5-Pentanediamine, -1-C¹⁴: 540
- , -1,5-C_{1/2}¹⁴: 280
- Pentanedioic acid, 2-amino-, -5-C¹⁴: 258
- 2,4-Pentanedione: 1389
- , H²-: 1604
- 1,3,4,5-Pentanetetrol, 1-(2-benzimidazolyl-2-C¹⁴)-: 1013
- 1,3,5-Pentanetrione, H²-1,5-diphenyl-: 1605
- Pentanoic acid, H²-3-hydroxy-: 1597
- , 2-(2-iodoacetamido)-5-methyl-, -I¹³¹: 1204
- 1-Pentanol, -1-C¹⁴ (also see Amyl alcohol): 870
- , -H²: 1343, 1456
- 2-Pentanol, -1,3-H₃²: 1330, 1344, 1485
- ; chlorosulfite ester: 1344
- 3-Pentanol, -H²-3-H²: 1346
- , -1,2-H₃²: 1347
- , -1,2-H₃²: 1514
- , -1,2,4,5-H₁₀²: 1347, 1516
- 2-Pentanone: 1344
- , -1-C¹⁴: 503, 645
- , -1-H₃²: 1513
- , -1,3-H₃²: 1344
- , 3-chloro-5-hydroxy-: 1117, 1118
- , 4,4-dimethyl-3-oxa-, -3-O¹⁸: 1873
- , 4-methyl-4-(methyl-H₃²-nitrosoamino)-: 1420
- 3-Pentanone: 1346, 1514, 1515
- , -3-C¹³: 646
- ; 2,4-dinitrophenylhydrazones: 646
- , -3-C¹⁴: 646, 647, 909
- , -2,4-C₂¹⁴: 647
- , -1-H₃²: 1513, 1514
- , -2,4-H₃²: 1515
- , -2,4-H₄²: 1412, 1514, 1515
- , -1,2-H₃²: 1514
- , -1,5-H₆²: 1516
- , -1,2,4-H₃²: 1514
- , -H₁₀²: 1515, 1516
- , 2-oxa-5-phenyl-, -3-O¹⁸: 1886
- Pentaquine, N₁¹⁵-: 1814, 1817, 1820
- ; monophosphate: 1818, 1820
- 1-Pentene, H²-: 1621
- 2-Pentene, -1,3-H₄²: 1345
- 2-Pentenoic acid, H²-: 1596
- 3-Pentenoic acid, H²-: 1596
- 4-Pentenoic acid, 5-amino-2,2-diethyl-3-oxo-, -5-C¹⁴; ethyl ester: 717
- , 2,2-diethyl-5-hydroxy-3-oxo-, -5-C¹⁴; ethyl ester: 717
- , 2,4,5-tribenzamido-; methyl ester: 1983
- 3-Penten-2-one, 4-(anilino-2,4,6-H₃²)-: 1389
- Pentobarbital, -2-C¹⁴; sodium derivative: 711
- , -N₁¹⁵: 1805
- ; sodium derivative: 1806
- Pentylamine, 5-chloro-*N*-isopropyl-; hydrochloride: 1814, 1820
- 2-Pentyne, -1-H₃²: 1513
- 1-Pentyn-3-ol; and L-: 1347
- ; 3,5-dinitrobenzoate: 1347
- ; hydrogen phthalate: 1347
- Perbenzoic acid: 915, 1558, 1576, 1702, 1882
- Performic acid: 943
- Perinaphthane: 858, 859
- Phenacetin, H²-: 1657
- Phenanthrene, -9-C¹⁴: 850
- ; picrate: 851
- , H²-: 1639
- , 3-methoxy-, -9,10-C_{1/2}¹⁴: 851
- , 1-methyl-, -9,10-C_{1/2}¹⁴: 684
- , 3-methyl-, -9,10-C_{1/2}¹⁴: 683
- 1-Phenanthreneacetic acid, 2-carboxy-1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-2-methyl-, -C¹⁴; inner anhydride: 1092
- ; dimethyl ester: 1089
- 1-Phenanthreneacetyl chloride, 7-acetoxy-2-carbomethoxy-1,2,3,4,4a-, 4b,5,6,7,8,10,10a-dodecahydro-2,4b-dimethyl-: 1096
- 2-Phenanthreneacetyl chloride, 1,2,3,4-tetrahydro-2-methyl-1-oxo-: 664
- 2-Phenanthrenecarboxylic acid, 7-acetoxy-1-(3-diazo-2-oxopropyl-3-C¹³)-1,2,3,4,4a,4b,5,6,7,8,10,10a-dodecahydro-2,4b-dimethyl-; methyl ester: 1096

- 1-Phenanthrenemalonic acid, 2-carboxy-1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-2-methyl-, $-C^{14}$: 1090
- 1-Phenanthrenepropionamide, 2-carbomethoxy-1,2,3,4,4a,4b,5,6,7,8,10, 10a-dodecahydro-7-hydroxy-2,4b-dimethyl-, $-\alpha-C^{13}$; acetate: 1095
- 1-Phenanthrenepropionic acid, 2-carboxy-1,2,3,4,4a,4b,5,6,7,8,10, 10a-dodecahydro-7-hydroxy-2,4b-dimethyl-, $-\alpha-C^{13}$: 1096
- , 2-carboxy-1,2,3,4,4a,9,10,10a-octahydro-7-methoxy-2-methyl-, $-\alpha-C^{14}$; dimethyl ester: 1092
- ; lead salt: 1092
- 1-Phenanthrenepropionic acid, 2-carboxy-1,2,3,4,4a,4b,5,6,7,8,10, 10a-dodecahydro-3 β -hydroxy-2,4b-dimethyl-, $-\alpha-C^{13}$; acetate, inner anhydride: 1096
- Phenanthrenequinone, $-9-C^{14}$: 681
- , 1-chloro-, $-9-C^{14}$: 681
- , 2-chloro-, $-9-C^{14}$: 680
- , 3-chloro-, $-9-C^{14}$: 681
- , 7-chloro-, $-9-C^{14}$: 680, 681
- , 1-methyl-, $-9,10-C^{14}_2$: 684
- , 3-methyl-, $-9,10-C^{14}_2$: 683
- 3-Phenanthrol, $-9,10-C^{14}_2$: 851
- 9-Phenanthrol, $-9-C^{14}$; acetate: 681
- , 1-chloro-, $-9-C^{14}$; and acetate: 681
- , 2-chloro-, $-9-C^{14}$; acetate: 679
- , 3-chloro-, $-9-C^{14}$; acetate: 681
- , 7-chloro-, $-9-C^{14}$; acetate: 681
- 1(2H)-Phenanthrone, 2-acetonyl-3,4-dihydro-2-methyl-: 664
- Phenethyl alcohol, $-\alpha-C^{14}$: 884, 907
- ; carbanilate: 907
- , $-\beta-C^{14}$: 76
- , $-\alpha, \beta-C^{14}_2$: 919
- ; acetate: 920
- ; carbanilate: 920
- , α -ethyl-1- C^{14} - β -ethyl- α -(*p*-methoxyphenyl)-*p*-methoxy-: 958
- , *p*-methoxy-, $-\alpha, \beta-C^{14}_2$: 921
- , *p*-nitro-, $-\alpha, \beta-C^{14}_2$: 921
- Phenethylamine, $-\alpha-C^{14}$: 519, 919, 921
- ; hydrochloride: 921
- , β -(4-biphenyl)-, $-\alpha-C^{14}$ (also see Phenethylamine, *p, \beta*-diphenyl): 745
- , 3,4-dihydroxy-, $-\alpha-C^{14}$; hydrochloride: 521
- , 3,4-dimethoxy-, $-\alpha-C^{14}$: 521
- , 3,4-dimethoxy-, $-\beta-C^{14}$: 521
- , 3,4-dimethyl- β -phenyl-, $-\alpha-C^{14}$; hydrochloride: 515
- ; nitrite: 515
- ; picrate: 515
- , *p, \beta*-diphenyl-, $-\alpha-C^{14}$: 514
- ; hydrochloride: 514, 745
- ; picrate: 514
- , 4-hydroxy-, $-\alpha-C^{14}$; and picrate: 226
- , β -mesityl-, $-\alpha-C^{14}$: 744
- , *p*-methoxy-, $-\alpha-C^{14}$: 520, 921
- ; hydrochloride: 921
- , β -(*p*-methoxyphenyl)-, $-\alpha-C^{14}$ (also see Phenethylamine, *p*-methoxy- β -phenyl-): 515, 745
- , *p*-methoxy- β -phenyl-, $-\alpha-C^{14}$; and hydrochloride: 515
- ; picrate: 515
- , α -methyl-, $-\beta-C^{14}$; sulfate: 513
- , *m*-methyl- β -phenyl-, $-\alpha-C^{14}$: 515
- ; hydrochloride: 515
- ; picrate: 515
- , *o*-methyl- β -phenyl-, $-\alpha-C^{14}$; hydrochloride: 515
- ; picrate: 515
- , *p*-methyl- β -phenyl-, $-\alpha-C^{14}$: 515
- ; hydrochloride: 515
- ; nitrite: 515
- ; picrate: 515
- , *p*-nitro-, $-\alpha-C^{14}$: 519, 921
- , β -*m*-tolyl-, $-\alpha-C^{14}$ (also see Phenethylamine, *m*-methyl- β -phenyl-): 515, 745
- , β -*o*-tolyl-, $-\alpha-C^{14}$ (also see Phenethylamine, *o*-methyl- β -phenyl-): 515
- , β -*p*-tolyl-, $-\alpha-C^{14}$ (also see Phenethylamine, *p*-methyl- β -phenyl-): 515, 745
- , 3,4,5-trimethoxy-, $-\alpha-C^{14}$: 522
- ; picrate: 523
- , 2,4,6-trimethyl- β -phenyl-, $-\alpha-C^{14}$; hydrochloride: 515
- ; nitrite: 515
- ; picrate: 515
- Phenethyl bromide, $-\alpha, \beta-C^{14}_2$: 884
- Phenethyl chloride, $-\alpha-C^{14}$: 884
- , $-\alpha, \beta-C^{14}_2$: 884
- o*-Phenetidine: 545
- Phenetole, H^2 : 1653
- , $-4-H^2$: 1380
- , 4-bromo-: 1380
- Pheniodol, I^{131}_2 : 1199
- Phenol: 38, 419, 668, 733, 1182, 1326
- ; acetate: 1341
- ; sodium salt: 365
- , $-1-C^{14}$: 323
- , $-1,2-C^{14}_2$: 539
- , $-C^{14}_4$: 324
- , H^2 : 1652
- , $-2,4,6-H^2_3$: 1322
- , $-O^{18}$: 1895
- , *o*-allyl-, $-H^2-4,6-H^2$: 1322

Phenol (*Continued*)

- , *o*-allyl-, -4,6- H_2^1 ; acetate: 1323
- , *o*-allyl-1- C^{14} —: 736, 739
- , 2-allyl-1- C^{14} -6-allyl—: 740
- , 2-allyl-1- C_1^{14} -4-allyl-3- C_1^{14} -6-allyl—: 740
- , 4-allyl-2,6-dimethyl-, - H^2 : 1527
- , *m*-amino—: 370
- , *p*-(2-aminoethyl-2- C^{14})—; and picrate: 226
- , 2-amino-5-nitro—: 367
- , 4,4'-[1,2-bis(ethyl-1- H_1^2)ethylene]di—; *meso*—: 1529
- , 4,4'-[1,2-bis(ethyl-1- H_1^2)ethylene]di—: 1692
- , 4,4'-[1,2-bis(ethyl-1- H_1^2)ethylene- H_2^3]di—: 1690
- , H^2 -*p*-chloro—: 1652
- , 2,4-dichloro—: 321
- , 5-diethylamino-2-nitroso—: 1226
- , 4,4'-(diethylideneethylene)di—: 1690
- , I^{131} -4,6-diiodo—: 1257
- , 2,2'-methylene- C^{14} -bis[3,4,6-trichloro—: 957
- , H^2 -*m*-nitro—: 1652
- , H^2 -*o*-nitro—: 1652
- , *p*-nitro—: 1910, 1922
- ; sodium salt: 1925, 1988, 1990
- , H^2 -*p*-nitro—: 1652
- , 4,4'-(tetrabromophthalidylidene)di—: 2003
- , 2,4,5-trichloro—: 957
- , I^{131} -2,3,6-triiodo—: 1257
- Phenolphthalein: 1202
- , 3',3'',5',5''-tetraiodo—, - I^{131} : 1202
- 1-Phenol-2-sulfonic acid, 4,4'-(tetrabromophthalidylidene)bis—, - S_2^{35} ; disodium salt: 2003
- Phenoltetrabromophthalein: 2003
- Phenothiazine, 2-chloro-10-(3-dimethylamino- C^{14} -propyl)—; hydrochloride: 532
- , 2-chloro-10-(3-methylaminopropyl)—; hydrochloride: 532, 533
- o*-Phenylenediamine: 789, 997, 1013
- ; dihydrochloride: 788, 927
- p*-Phenylenediamine, *N,N*-dimethyl-, - N^1 - N^{15} : 1725
- Phloretic acid, 3,5-diiodo- α -phenyl-, - I_2^{131} : 1199
- , α -phenyl—: 1199
- Phloroglucinol, H^2 —: 1653
- Phosgene: 600, 715, 1180, 1905, 1906
- , - C^{11} : 595
- , - C^{14} : 340, 484, 586, 587, 602
- Phosphine, bis(trifluoromethyl)iodo—: 1500
- , trifluoromethyldiiodo—: 1500
- , tris(trifluoromethyl)—: 1500
- Phosphine oxide, benzyldiphenyl-, - P^{32} : 1900
- , butylbis(*m*-nitrophenyl)—, - P^{32} : 1900
- , butyldiphenyl-, - P^{32} : 1900
- , ethyldiphenyl-, - P^{32} : 1899
- , isobutyldiphenyl-, - P^{32} : 1900
- , isopropyldiphenyl-, - P^{32} : 1900
- , methyldi-*p*-tolyl-, - P^{32} : 1900
- Phosphine sulfide, tris(1-aziridinyl)—, - C_6^{14} : 408
- , tris(1-aziridinyl)—, - H_{12}^3 : 1708
- Phosphinic acid, bis(2-ethylhexyl)—, - P^{32} : 1901
- , diphenyl-, - P^{32} : 1899
- , di-*p*-tolyl-, - P^{32} : 1899, 1900
- Phosphinic chloride, bis(2-ethylhexyl)—, - P^{32} : 1901
- , diphenyl-, - P^{32} : 1899
- Phosphonic acid, ethyl-, - P^{32} ; diethyl ester: 1904
- Phosphonic dichloride, ethyl-, - P^{32} : 1902
- , (2-ethylhexyl)—, - P^{32} : 1902
- , 1-piperidyl—: 1902
- Phosphonium iodide, H^2 -tetramethyl—: 1651
- Phosphonous dichloride, ethyl-, - P^{32} : 1902
- Phosphoric acid; acetyl dibenzyl ester: 1890
- ; monoacetyl ester, disilver salt: 1889
- ; monoacetyl-1- C^{13} ester, disilver salt: 474
- , - O_1^{18} ; and dimethyl ester: 1889
- , - P^{32} : 1904, 1906, 1907, 1909, 1917
- , - P^{32} ; calcium hydrogen salt: 1915
- , - P^{32} ; calcium salt: 1906, 1915
- , - P^{32} ; dibutyl ester: 1913
- , - P^{32} ; 2,3-dimethyl-1,3-dioxolane-4-methyl ester, barium salt: 1908
- , - P^{32} ; dipotassium salt: 1907
- , - P^{32} ; disodium salt: 1906
- , - P^{32} ; ferric salt: 1904
- , - P^{32} ; mono-2,3-dihydroxypropyl ester, barium salt: 1909
- , - P^{32} ; mono-2-hydroxypropyl ester, barium salt: 1907
- , - P^{32} ; mono-2-hydroxypropyl ester, lead salt: 1907
- , - P^{32} ; mono-2-hydroxypropyl ester, sodium salt: 1908
- , - P^{32} ; mono-*p*-nitrophenyl ester: 1910
- , - P^{32} ; mono-*p*-nitrophenyl ester, barium salt: 1911

- , $-P^{32}$; mono-*p*-nitrophenyl ester, sodium salt: 1910
- , $-P^{32}$; potassium dihydrogen salt: 1915
- , $-P^{32}$; silver salt: 1906, 1919, 1927
- , $-P^{32}$; tributyl ester: 1913, 1919
- Phosphoric acid- H_3^+ : 1285, 1337, 1364, 1377, 1386
- Phosphoric anhydride, bis ($-P^{32}$): 1927
- Phosphorochloridic acid, $-P^{32}$; diethyl ester: 1914, 1924
- , $-P^{32}$; diisopropyl ester: 1916, 1918
- Phosphorochloridothionic acid, $-P^{32}$; diethyl ester: 1923, 1925
- ; dimethyl ester: 1922
- ; mono-*p*-nitrophenyl ester: 1925
- , $-P^{32}-S^{35}$; diethyl ester: 1988
- , $-S^{35}$; diethyl ester: 1990
- Phosphorodiamidic acid, tetramethyl-ethyl ester: 1921
- Phosphorodiamidic anhydride, bis (tetramethyl-, $-P^{32}$): 1921
- Phosphorodiamidic chloride, tetramethyl-, $-P^{32}$: 1920
- Phosphorofluoridic acid, $-P^{32}$; diisopropyl ester: 1916, 1918
- Phosphorothiolic acid; diethyl *S*-[2-(ethylthio)ethyl] ester: 1923, 1924
- Phosphorothionic acid, $-P^{32}$; diethyl 2-(ethylthio)ethyl ester: 1923, 1924
- ; diethyl *p*-nitrophenyl ester: 1925, 1926
- ; dimethyl *p*-nitrophenyl ester: 1922
- ; trimethyl ester: 1922
- , $-P^{32}-S^{35}$; diethyl *p*-nitrophenyl ester: 1988
- , $-S^{35}$; diethyl *p*-nitrophenyl ester: 1990
- Phosphorous acid, $-P^{32}$; diisopropyl ester: 1916, 1918
- ; triethyl ester: 1904, 1914
- Phosphorus, $-P^{32}$: 1917, 1924
- Phosphorus pentachloride, $-P^{32}$: 1915, 1918, 1924
- Phosphorus pentasulfide- S_5^{35} : 1940, 1991
- Phosphorus trichloride, $-P^{32}$: 1902, 1904, 1906, 1914, 1916, 1917, 1923, 1924, 1988
- Phosphoryl chloride, $-P^{32}$: 1901, 1904, 1905, 1906, 1908, 1910, 1912, 1915, 1920, 1927
- σ -Phthalaldehydic acid, $-C^{14}$: 791
- Phthalazine, 1-chloro-, $-1-C^{14}$: 792
- , 1-hydrazino-, $-1-C^{14}$: 792
- ; dihydrochloride: 792
- ; hydrochloride: 792
- 1-Phthalazinesulfonic acid, 4-carboxymethyl-3,4-dihydro-3-(*p*-nitrophenyl)-, $-2-N^{15}$; sodium salt: 1823
- 1-Phthalazinol, 4-carboxymethyl-3,4-dihydro-3-(*p*-nitrophenyl)-, $-2-N^{15}$: 1823
- Phthalazinium hydroxide, 2-(*p*-aminophenyl)-4-hydroxy-1-methyl-, $-3-N^{15}$; inner salt: 1824
- , 4-hydroxy-1-methyl-2-(*p*-nitrophenyl)-, $-3-N^{15}$; inner salt: 1824
- Phthalazinium sulfate, 4-hydroxy-1-methyl-2-(*p*-nitrophenyl)-, $-3-N^{15}$: 1823
- 1 (2*H*)-Phthalazinone, $-1-C^{14}$: 791
- , 2-(*p*-acetamidophenyl)-4-methyl-, $-3-N^{15}$: 1826
- , 2-(*p*-aminophenyl)-4-methyl-, $-3-N^{15}$: 1826
- , 4-methyl-2-(*p*-nitrophenyl)-, $-3-N^{15}$: 1826, 1827
- , 4-methyl-3-(*p*-nitrophenyl)-, $-3-N^{15}$; sulfate: 1827
- , 4-methyl-2-phenyl-, $-3-N^{15}$: 1826
- Phthalic acid: 1731
- ; bis (2-ethylhexyl- $-1-C^{14}$) ester: 432
- ; calcium salt: 1440
- ; monoammonium- N^{15} salt: 1731
- , $-C^{14}$: 439, 744, 848, 849, 851
- ; dianilide: 851
- ; dimethyl ester: 440
- , $-3-C^{14}$: 1146
- , $-1,2-C^{14}$: 414
- ; mercuric salt: 414
- , O^{18} —: 1896
- Phthalic acid- H_3^+ : 1287
- Phthalic anhydride: 240, 1287, 1328
- , $-C^{14}$: 848, 849
- , 3,6-*endomethylene*-1,2,3,6-tetrahydro-, $-C^{14}$; *cis*—: 428
- Δ^4 -tetrahydro-, $-1-C^{14}$; *cis*—: 414
- Δ^4 -tetrahydro-, $-1,2-C^{14}$; *cis*—: 414
- Phthalide, 3,3-bis (4-hydroxy-3,5-diiodophenyl)-, $-1-I^{131}$: 1202
- Phthalimide; potassium derivative: 239, 453, 491, 495
- , $-N^{15}$: 1731, 1740, 1741
- ; potassium derivative: 1719, 1731, 1737, 1750, 1751, 1766, 1777, 1779, 1782, 1785, 1788, 1792, 1816, 1835, 1860
- , *N*-(2-bromoethyl)-, $-N^{15}$: 1835
- , *N*-(2-bromoethyl- $-C^{14}_{1/2}$)-: 495
- , *N*-(3-bromopropyl)-: 1780

- Phthalimide (*Continued*)
 —, *N*-(3-bromopropyl)-, -N¹⁵: 1782
 —, *N*-(5-bromopentyl)-, -N¹⁵: 1816
 —, *N*-(chloromethyl)-: 160, 1742
 —, *N*-(chloromethyl)-, -N¹⁵: 176, 1742
 —, *N*-(cyano-C¹³-methyl)-, -N¹⁵: 1742
 —, *N*-(cyano-C¹⁴-methyl)-, -N¹⁵: 176
 —, *N*-(3-cyanopropyl)-: 507
 —, *N,N'*-(ethylene-C¹⁴)di-: 495
 —, *N*-(hydroxymethyl)-: 162
 —, *N*-(hydroxymethyl)-, -N¹⁵: 1741
 —, *N*-(4-iodobutyl)-: 280, 281
 —, *N*-(3-iodopropyl)-: 280
 —, *N*-isobutyryl-: 1313
 —, *N*-methyl-C¹³-: 491
 —, *N,N'*-pentamethylenebis(-, -N¹⁵): 1816
 —, *N*-phenyl-, -C¹⁴: 744
 Phthaloyl chloride: 376
 —, -C¹⁴: 440
 Phyllanthane: 1585
 Phyllanthol: 1585
 —; acetate: 1586
 4-Picoline, -2-C¹⁴; and sulfate: 113
 —, 2,3,6-trichloro-, -2,6-C¹⁴₂: 113
 Pimelic acid, -1,7-C¹⁴: 823
 Pimelonitrile, -C¹⁴: 823, 826
 Pinacolone: 910
 —, C¹⁴₂-: 648
 4-Pipecoline: 114
 Piperazine, 1-methyl-, -2,6-C¹⁴; dihydrochloride: 403, 404
 —; dipicrate: 405
 1-Piperazinecarboxamide, *N,N*-diethyl-4-methyl-, -3,5-C¹⁴; dihydrogen citrate: 404
 2,5-Piperazinedione, -3,6-C¹⁴: 718
 —; hydrochloride: 719
 —, O¹⁸-: 1894
 —, 3,6-bis[2-(3-amino-3-carboxypropylthio)ethyl]-, -S³⁵: 1964
 —, 3,6-bis[2-(benzylthio)ethyl]-, -S³⁵: 1958
 —, 3,6-bis(2-chloroethyl)-: 1958, 1964, 1972
 —, 3,6-bis(hydroxymethyl-C¹⁴)-: 173
 2,6-Piperazinedione, 4-methyl-, -3,5-C¹⁴: 403
 3,5-Piperazinedione, 1-methyl-, -2,6-C¹⁴: 403
 Piperidine: 1386
 —, -1-H²: 1386
 —, 1-(*o*-nitrophenyl)-; hydrochloride-H²: 1387
 —, 1-(*p*-nitrophenyl)-; hydrochloride-H²: 1387
 2,4-Piperidinedione, 3,3-diethyl-, -6-C¹⁴: 718
 —, 3,3-diethyl-5-methyl-, -6-C¹⁴: 717
 2-Piperidone, -3,4,5,6-H²-N¹⁵: 1768
 —, -N¹⁵: 1783
 —, 3,3-dichloro-, -4,5,6-H²-N¹⁵: 1768
 Piperil: 1367
 Piperonal, -H²: 1367
 Piperonylic acid, -O¹⁸: 1872
 Pipsyl chloride, -I¹³¹: 1240
 Pivalic acid, -C¹⁴(also see Propionic acid, 2,2-dimethyl-): 96
 —, H²-: 1596
 —, -O¹⁸: 1880
 Platinum, bis(ethylenedichloro)-μ-dichlorodi-, (II): 1407
 Polyoxymethylene: 1968
 Polysaccharide: 1008, 1249
 —, I¹³¹-: 1249
 Porphyrin-6,7-dipropionic acid, 1,3,4,8-tetramethyl-2,4-bis(1-hydroxyethyl-1-C¹⁴)-; dimethyl ester: 471
 Potassium benzeneisodiazoate, -α-N¹⁵: 1863
 Potassium carbonyl-C¹⁴: 1049
 Potassium, 1,1-diphenylethyl-: 1452
 —, triphenylmethyl-: 1429
 Potassium hydrosulfide: 1939
 Potassium hydrosulfide-S³⁵: 1935, 1936
 Potassium permanganate-O¹⁸: 1871, 1872
 Potassium triiodide-I¹³¹: 1205
 4,16-Pregnadiene-3,20-dione: 1561
 —, H²-: 1561
 9,17-Pregnadiene-3α,11,20-triol-, -4,16-21-H²; triacetate: 1702
 5,17-Pregnadien-3-one, 20-hydroxy-4-oxa-; acetate: 1066
 Pregnane-3α,20-diol, 17α,20-epoxy-, -11,12-H²; diacetate: 1576
 3,20-Pregnanedione, -11,12-H²: 1573
 —, 4-bromo-, -11,12-H²: 1573
 —, 4-bromo-17α-hydroxy-: 1578
 —, 4-bromo-17α-hydroxy-, -11,12-H²: 1577
 —, 2,4-dibromo-, -11,12-H²: 1575
 —, 17α-hydroxy-, -11,12-H²: 1577
 11,20-Pregnanedione, 21-bromo-3α, 17α-dihydroxy-, -4,16,21-H²: 1702
 —, 3α,17α-dihydroxy-, -4,16,21-H²: 1697, 1698, 1702
 —; 3-acetate: 1698
 —, 3α-hydroxy-; acetate: 1701
 —, 3α-hydroxy-, -4,16,21-H²: 1699, 1701
 —, 3α,17α,21-trihydroxy-, -4,16,21-H²: 1702
 3,11,20-Pregnanetrione, -4,16,21-H²: 1699

- , 4-bromo-17 α ,21-dihydroxy-, -4,16,-21-H₃²; 21-acetate: 1703
- , 17 α ,21-dihydroxy-, -4,16,21-H₃²; and 21-acetate: 1703
- 20 α -Pregnanol; acetate-H₃²: 1545
- 20-Pregnanone, 3 α ,17 α -dihydroxy-; and 3-acetate: 1578
- , 3 α ,17 α -dihydroxy-, -11,12-H₂²: 1577
- , 3 α -hydroxy-, -11,12-H₂²: 1558, 1559, 1566, 1573
- ; formate: 1558
- 9-Pregnene-3 α ,11,20-triol, 17 α ,20-epoxy-, -4,16,21-H₃²; triacetate: 1702
- 4-Pregnene-3,11,20-trione, 17 α ,21-dihydroxy-, -4,16,21-H₃²; 21-acetate: 1703
- Pregnenolone, C¹⁴-; acetate: 1075
- Pregnenone, C¹⁴-hydroxy-; acetate: 1075
- 5-Pregnen-20-one, C¹⁴-21-diazo-3 β -hydroxy-; and acetate: 1065
- , C¹⁴-3 β ,21-dihydroxy-; 21-acetate: 1065
- , 3 β -hydroxy-: 1567, 1572
- ; acetate-H₃²: 1545
- , 3 β -hydroxy-, -21-C¹⁴; and acetate: 1074
- , 3 β -hydroxy-, -21-H₃²; acetate: 1568
- , 3 β -hydroxy-, -17,21-H₄²; and acetate: 1569
- ; acetate-H₃²: 1545, 1569
- , 3 β -hydroxy-H²-, -17,21-H₄²: 1569
- , C¹⁴-3 β -hydroxy-: 1076, 1083
- ; acetate: 1075
- ; acetate, digitonide: 1076
- Procaine: 1151
- , C¹⁴-: 457
- , C¹⁴-; hydrochloride: 456
- , dibromo-, -Br⁸²; and hydrochloride: 1151
- ; hydrobromide: 1151
- Proflavine; dihydrochloride: 1679
- Progesterone: 1575
- , C¹⁴-: 1076
- , -3-C¹⁴: 1066, 1067
- , -4-C¹⁴: 1063, 1070, 1072
- , -21-C¹⁴: 1073, 1074
- , H²-: 1575
- , -11,12-H₃²; and mono-2,4-dinitrophenylhydrazones: 1574
- , H²-16-dehydro-: 1561
- , 21-diazo-, -3-C¹⁴: 1061, 1067
- , 21-diazo-, -4-C¹⁴: 1063, 1070
- , 17 α -hydroxy-, -11,12-H₂²: 1577
- , C¹⁴-21-hydroxy-; acetate: 1065
- , H²-16 α -methoxy-: 1561
- 17 α -Progesterone, H²-: 1575
- Proline; copper salt: 241
- , C¹⁴-: 240
- , H²-; L(-)-: 1634
- , -3,4,5-H₃²-N¹⁵; and copper salt: 1768
- , -N¹⁵; and hydrochloride: 1770
- , 4-allohydroxy-: 1772
- , 4-allohydroxy-, -2-C¹⁴: 243
- ; bis-2-naphthalenesulfo-derivative: 243
- ; copper salt: 243
- , 4-hydroxy-, -2-C¹⁴: 242
- ; copper salt: 243
- ; 2-naphthalenesulfo-derivative: 243
- , 4-hydroxy-, -N¹⁵: 1771
- ; ammonium-N¹⁵ salt: 1772
- ; copper salt: 1772
- , H²-hydroxy-: 1634
- Propadiene, -H₄²: 1427
- , 1-(1-naphthyl)-1,3-diphenyl-3-phenyl-H₂²: 1462, 1463
- Propane, -1-C¹³: 804, 805, 806
- , -2-C¹³: 805
- , -1,2-C¹³₂: 806
- , H²-: 1611
- , -1-H₁²: 1403, 1404
- , -2-H₁²: 1403, 1404
- , -2-H₂²: 1405
- , -1,3-H₂²: 1408
- , -H₂²: 1409
- , 2-benzyl-1-bromo-: 97
- , 1,3-bis(1,3-dioxo-2-isoindolyl)-, -N¹⁵: 1782
- , 1-bromo-: 95, 96
- , 1-bromo-, -Br⁸²: 1171
- , 1-bromo-, -1-C¹³: 805
- , 1-bromo-, -1-C¹⁴: 807, 869
- , 1-bromo-, -2-C¹⁴: 65
- , 1-bromo-, -1-H₂²: 1522
- , 2-bromo-: 95
- , 2-bromo-, -Br⁸²: 1172
- , 2-bromo-, -1-C¹³: 37
- , 2-bromo-, -1-C¹⁴: 37, 910, 918
- , 2-bromo-, -2-C¹⁴: 37, 605
- , 2-bromo-, -2-H²: 1332
- , 2-bromo-, -1,3-H₂²: 1332, 1432
- , 2-bromo-, -H₂²: 1331
- , 1-bromo-3-chloro-: 451
- , 1-bromo-2-methyl-: 96
- , 1-bromo-2-methyl-, -Br⁸²: 1173
- , 1-bromo-2-methyl-, -1-C¹⁴: 217, 870
- , 1-bromo-2-methyl-, -2-C¹⁴: 217
- , 2-bromo-2-methyl-, -Br⁸⁰ (also see *t*-Butyl bromide): 1173
- , 2-bromo-2-methyl-, -Br⁸²: 1173
- , 1-bromo-3-phenyl-: 97
- , 1-bromo-3-phenyl-, -3-H₁²: 1457
- , 1-chloro-, -Cl³⁶: 1191

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- , 2-chloro-, -2-H²: 1405, 1495
- , 2-chloro-, -1,3-H₂²: 1408
- , 2-chloro-, -H₂²: 1409
- , 1-chloro-2,2-dimethyl-: 1413
- , 1-chloro-2-methyl-, -1-H₂²: 1399
- , 2-chloro-2-methyl-, -2-C¹³ (also see *t*-Butyl chloride): 811
- , 2-chloro-2-methyl-, -2-C¹⁴: 879, 930
- , 2-chloro-2-methyl-, -Cl³⁶: 1185, 1191
- , 1,2-dibromo-, -3-H₂²: 1426, 1483
- , 1,3-dibromo- (also see Trimethylene bromide): 1770
- , 2,2-dibromo-: 1405
- , 2,2-dimethyl-, -H₂²: 1413
- , H²-2,2-dimethyl-: 1613
- , 1,1,1,2,2,3,3-heptachloro-, -3-C¹⁴: 873
- , 1-iodo-: 43
- , 1-iodo-, -1-C¹³: 37, 804, 806
- , 1-iodo-, -1-C¹⁴: 57, 43, 197, 869
- , 1-iodo-, -I¹²⁸: 1254
- , 1-iodo-, -I¹³¹: 1254
- , 2-iodo-: 1404, 1817
- , 2-iodo-, -1,3-C₂¹⁴: 196, 870, 910
- , 2-iodo-, -I¹²⁸: 1254
- , 1-iodo-2-methyl-, -1-C¹⁴: 216, 870, 876
- , 1-iodo-2-methyl-, -I¹²⁸: 1255
- , 2-iodo-2-methyl-, -1-C¹³: 810
- , 2-iodo-2-methyl-, -I¹²⁸: 1255
- , 2-methyl-, -1-C¹³: 810
- , 2-methyl-, -2-C¹³: 811
- , 2-methyl-, -1-C¹⁴: 810
- , 2-methyl-, -2-H²: 1405, 1410, 1613
- , 2-methyl-, -1-H₂²: 1613
- , 2-methyl-, -2-H³: 1683
- , 2-methyl-, -1-H₂³: 1683
- , H²-2-methyl-: 1612, 1613
- , H³-2-methyl-: 1682
- , 1,1,2,2-tetrachloro-, -H₂²: 1496
- , 1,2,2,3-tetrachloro-, -H₂²: 1496
- 1,3-Propanediamine, -1-C¹⁴; dipicrate: 264
- 1,1-Propanedicarboxylic acid, 3-carboxy-C¹⁴-2-methyl- (also see Glutaric acid, 2-carboxy-3-methyl-): 136
- 1,2-Propanediol: 1908
- ; carbonate: 1338
- , -1-C¹⁴: 929
- , -2-C¹⁴; 1-monophosphate: 475
- , 3-benzyloxy-, -1-C¹⁴: 938
- , 3-(*o*-methoxyphenyl)-, -3-C¹⁴: 736
- , 3-(6-methoxy-*m*-tolyl)-, -3-C¹⁴: 623
- , 3-(4-methoxy-3,5-xylyl)-, -1-C¹⁴: 735
- , 3-(4-methoxy-3,5-xylyl)-, -1,3-C_{1/2}¹⁴: 738
- , 2-methyl-, -2-C¹⁴: 930
- , 3-phenoxy-, -1-C¹⁴: 734
- , 3-*p*-tolxyloxy-, -1-C¹⁴: 732
- , 3-(2,6-xylyloxy)-, -1-C¹⁴: 734
- 1,3-Propanediol, 2-amino-; oxalate: 641
- , 2-amino-, -1,3-C₂¹⁴; dihexanoate, hydrochloride: 935
- ; hydrochloride: 935
- ; oxalate: 935
- , 2-amino-1-*p*-nitrophenyl-; D-, *threo*-: 385
- ; L-: 385
- ; L-, dibenzoyltartrate: 384
- , 2-amino-1-*p*-nitrophenyl-, -3-C¹⁴: 384
- ; D- and L-, *threo*-: 384
- ; L-dibensoyltartrate: 384
- , 2-(hydroxyamino)-2-(hydroxymethyl)-, -2-C¹⁴: 638
- , 2-(hydroxyamino)-2-(hydroxymethyl-C¹⁴)-, -1,3-C₂¹⁴: 639
- , 2-(hydroxymethyl)-2-nitro-, -2-C¹⁴: 638
- , 2-(hydroxymethyl-C¹⁴)-2-nitro-, -1,3-C₂¹⁴: 639
- , 2-nitro-, -2-C¹⁴: 641
- ; 2-sodium derivative: 639
- , 2-nitro-, -1,3-C₂¹⁴; 2-sodium derivative: 934
- 1,2-Propanedione, 1,3-diphenyl-, -1-C¹⁴: 670
- 1,3-Propanedione, 2-bromo-1,3-diphenyl-, -1-C¹⁴: 642
- , 2-bromo-2-hydroxy-1-(*p*-methoxyphenyl)-3-phenyl-, -1-C¹⁴; acetate: 643
- , 2-bromo-1-(*p*-methoxyphenyl)-3-phenyl-, -1-C¹⁴: 643
- , 1,3-diphenyl-: 1342
- , 1,3-diphenyl-, -1-C¹⁴: 642
- , 2-hydroxy-1,3-diphenyl-, -1-C¹⁴; acetate: 642
- , 3-(2-hydroxy-4-methoxyphenyl)-1-phenyl-, -1-C¹⁴: 701
- , 3-[2-hydroxy-4-(tetrahydropyran-2-yloxy)phenyl]-1-mesityl-, -1-C¹⁴: 701
- , 3-[2-hydroxy-4-(tetrahydropyran-2-yloxy)phenyl]-1-phenyl-, -1-C¹⁴: 699
- , 1-(*p*-methoxyphenyl)-3-phenyl-, -1-C¹⁴: 643
- 1,2,3-Propanetriol, -1-C¹⁴: 932, 933
- , -2-C¹⁴: 932, 936
- , -1,3-C₂¹⁴: 437, 936
- , 1-H₂²: 1352

- , 1,2-isopropylidene-, -H²-3-H²; D-: 1352
- , 1-(*p*-methoxyphenyl)-3-phenyl-, -1-C¹⁴; 2-acetate: 643
- Propanetrione, 1,3-diphenyl-, -1-C¹⁴: 150, 642
- , 1-(*p*-methoxyphenyl)-3-phenyl-, -1-C¹⁴: 149, 643
- 1-Propanol: 806
- , -1-C¹³: 37, 804, 805, 806
- ; benzoate: 805
- , -1-C¹⁴: 500, 805, 807, 808, 868, 909, 910, 929
- , -2-C¹⁴: 65, 805, 929
- , -1,2-C¹⁴₂: 500
- ; 3,5-dinitrobenzoate: 500
- , -2,3-C¹⁴₂: 910
- , -H²: 1344
- , -1-H²: 1521
- , 3-chloro-: 964
- , 3-chloro-, -1-C¹⁴: 731, 733, 734
- , 2,3-dibromo-: 1936, 1937
- , 2,3-dichloro-: 1184
- , 2,3-dichloro-, -3-C¹³: 1183
- , 2,3-dimercapto-, -S³⁵: 1936, 1937
- , 2,2-dimethyl-, -O¹⁸: 1880
- , 3-(ethylthio)-: 93
- , 1-methyl-C¹⁴ (also see 2-Butanol); carbanilate: 918
- ; *p*-toluenesulfonate: 506, 917
- , 2-methyl-, -1-C¹⁴: 809, 870, 875, 909
- , 2-methyl-, -3-C¹⁴: 910, 919
- , 2-methyl-, -H²: 1344
- , 3-phenoxy-, -1-C¹⁴: 733
- , 3-phenyl-, -3-H²: 1456
- , 3-*p*-tolylloxy-, -1-C¹⁴: 731
- , 3-(2,6-xylyloxy)-, -1-C¹⁴: 734
- 2-Propanol (also see Ethanol, 1-methyl-); aluminum derivative: 1334
- , -1-C¹⁴: 501, 633, 910, 918
- , -2-C¹⁴: 604, 605, 808
- , -1,3-C¹⁴₂: 870, 910
- , -H²: 1334, 1602
- , -H²-2-H²: 1333, 1495
- , -2-H²: 1332, 1333, 1348, 1495
- ; aluminum derivative: 1348
- ; chromate ester: 1333
- ; nitrate ester: 1332
- , -1,3-H²₂: 1332, 1403, 1482
- ; nitrate ester: 1332
- , -1,2,3-H²₃: 1331, 1409
- ; nitrate ester: 1331
- , -2-H³: 1670
- , 1,3-dichloro-: 602, 938, 1184
- , 2-ethoxymethyl-, -2-C¹³: 192
- , 2-methyl-, -2-C¹⁴: 879, 910, 930
- , 2-methyl-, -O¹⁸: 1872
- ; chromate ester: 1873
- , H²-2-methyl-: 1602
- , 1,1,1-trichloro-2-methyl-C¹⁴-, -3-C¹⁴: 956
- 2-Propanone-, -2-C¹³: 635, 805
- , -1,3-C¹³₂: 39
- , -1-C¹⁴: 633, 644
- ; 2,4-dinitrophenylhydrazone: 633, 877
- ; semicarbazone: 634
- , -2-C¹⁴: 37, 55, 58, 604, 605, 634, 635, 785, 808, 834
- , -1,3-C¹⁴₂: 55, 467, 488, 634, 635, 870, 910
- ; 2,4-dinitrophenylhydrazone: 635
- ; *p*-nitrophenylsemicarbazone: 635
- , H²₂: 1512
- , -H²₂: 1511, 1512
- , -H²₂: 1388, 1397, 1482, 1498, 1510, 1511, 1512
- , (benzylthio)-: 209
- , 1-chloro-, -1-C¹⁴: 877
- , 3-chloro-1-hydroxy-, -3-C¹⁴; 1-hexadecanoate-1-C¹⁴: 455
- , 3-diazo-1-hydroxy-, -2-C¹⁴; acetate: 637
- , 3-diazo-1-hydroxy-, -3-C¹⁴; 1-hexadecanoate-1-C¹⁴: 455
- , 1,3-dichloro-: 156, 938
- , 1,3-dihydroxy-, -2-C¹⁴: 637, 638, 639
- ; copper complex: 640
- ; 2,4-dinitrophenylhydrazone: 641
- ; oxime: 638
- ; phenylosazone: 641
- , 1,3-dihydroxy-, -3-C¹⁴; 1-hexadecanoate-1-C¹⁴: 455
- , 1,3-dihydroxy-, -1,3-C¹⁴₂; and oxime: 639
- , methoxy-C¹⁴-, 697
- ; 2,4-dinitrophenylhydrazone: 697
- , 1-phenyl-, -1-C¹⁴: 512
- ; oxime: 513
- ; semicarbazone: 513
- , 1,1,3-tribromo-: 312
- Propene: 1404
- , C¹⁴-, 808
- , -1-C¹⁴: 807, 808
- , -2-C¹⁴: 808
- , -3-C¹⁴: 808
- , -1,3-C¹⁴₂: 808
- , H²-, 1620
- , -1-H²₁: 1427, 1483
- , -3-H²₁: 1426, 1482, 1483
- , -H²₂: 1404, 1427
- , 1-bromo-, -Br⁸⁰: 1170
- , 2-bromo-, -Br⁸⁰: 1170

Propene (*Continued*)

- , 3-bromo-, (also see Allyl bromide): 43, 95
- , 3-bromo-, -Br⁸⁰: 1170
- , 3-bromo-, -1,3-C₁¹⁴₂: 871
- , 1-chloro-2-methyl-, -3-C¹⁴: 877
- , 3-chloro-2-methyl-, -3-C¹⁴: 876
- , 1,2-dichloro-, -H₂²; *cis*:-: 1496, 1497
- ; *trans*:-: 1496
- , 1,3-dichloro-, -3-Cl³⁶; *cis*- and *trans*:-: 1184
- , hexachloro-, -1,3-C₁¹⁴₂: 873
- , 3-iodo-, -I¹²⁸: 1254
- , 3-iodo-, -I¹³¹: 1254
- , 2-methyl-, -2-C¹³: 811
- , 2-methyl-, -1,3-C₁¹³₂: 810
- , 2-methyl-, -1-C¹⁴: 376
- , 2-methyl-, -2-C¹⁴: 930
- , C₁¹⁴-2-methyl:-: 809
- , H²-2-methyl:-: 1621
- , 3-phenyl-, -3-H₂²: 1457
- , 1,1,2-trichloro-3,3,3-trifluoro-, -1,3-C₁¹⁴₂: 873
- 1-Propene-3-sulfonic acid, 3-hydroxy-1-phenyl-, -S³⁵; sodium salt: 1999
- 2-Propene-1-sulfonic acid, 1-hydroxy-3-phenyl-, -S³⁵; sodium salt: 1999
- 1-Propen-1-ol, 3-oxo-2,3-diphenyl-, -1-C¹⁴; benzoate: 625
- 2-Propen-1-ol, 3-chloro-; *cis*- and *trans*-(also see Allyl alcohol): 1184
- , 3-(3,4-dimethoxyphenyl)-, -1-C¹⁴: 1031
- , 3-(4-β-D-glucosyloxy-3,5-dimethoxyphenyl)-, -2-C¹⁴: 1035
- , 3-(4-β-D-glucosyloxy-3,5-dimethoxyphenyl)-, -3-C¹⁴: 1038
- , 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-, -1-C¹⁴: 1030, 1031
- ; L:-: 1031
- , 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-, -2-C¹⁴: 1034
- , 3-(4-β-D-glucosyloxy-3-methoxyphenyl)-, -3-C¹⁴: 1037, 1038
- , 3-(4-β-D-glucosyloxyphenyl)-, -2-C¹⁴: 1035
- , 3-(4-β-D-glucosyloxyphenyl)-, -3-C¹⁴: 1038
- , 3-(4-hydroxy-3-methoxyphenyl)-, -1-C¹⁴: 1029
- , 1-(1-naphthyl)-1,3-diphenyl-3-phenyl-H₂²:-: 1343, 1463
- , 3-(1-naphthyl)-1,3-diphenyl-1-phenyl-H₂²:-: 1462
- , 3-[4-(tetra-O-acetyl-β-D-glucosyloxy)-3-methoxyphenyl]-, -2-C¹⁴: 1034

- β-Propiolactone, O₁¹⁸-(also see Hydroxylic acid; β-lactone): 1874
- , 3-ethylidene-2-hexyl-, -3-C¹⁴: 482
- , 3-heptylidene-2-methyl-, -1-C¹⁴: 482
- Propionaldehyde: 917, 1514
- , -2,3-C₁¹⁴₂: 506
- , 2,3-dibromo-, -3-C¹⁴: 308
- , 2-methyl-, -2-C¹³: 193
- , 2-methyl-, -2,3-H₂²; diethyl acetal: 1295
- , 3-(methylthio)-, -1-C¹⁴: 213, 629
- ; dimethyl acetal: 629
- ; 2,4-dinitrophenylhydrazones: 630
- , 3-(methylthio)-, -S³⁵: 1953, 1955
- , 2-oxo-, -2-C¹⁴; disemicarbazones: 641
- , 2,2,3-tribromo-: 312
- Propionic acid: 91
- ; methyl ester: 1323
- , -1-C¹¹: 95
- , -1-C¹³: 804
- ; barium salt: 646
- ; dodecyl ester: 805
- ; sodium salt: 37
- , -2-C¹³: 37
- , -2-C¹³-1-C¹⁴: 704
- , -2-C¹³-3-C¹⁴: 43, 141
- , -3-C¹³: 37, 43
- , -1-C¹⁴: 91, 95, 114, 115, 164, 378, 379, 482, 500, 668, 805, 868, 910, 929
- ; cadmium nickel salt: 368
- ; propyl ester: 306, 808, 909
- ; sodium salt: 164, 331, 806, 868
- , -2-C¹⁴: 37, 43, 47, 65, 138, 805, 929
- ; *p*-bromophenacyl ester: 47
- ; sodium salt: 138, 667
- , -3-C¹⁴: 37
- ; ethyl ester: 418, 706
- ; sodium salt: 418
- , -1,2-C₁¹⁴₂; and *p*-toluidide: 501
- , -2,3-C₁¹⁴₂: 506
- , H²:-: 1595
- ; ethyl ester: 1643
- , -2-H₂²; methyl ester: 1323
- , -2-H₂²: 1421
- ; silver salt: 1473
- , -2,3-H₂²: 1293
- , -3-H₂²: 1264, 1265, 1324, 1422
- ; ethyl ester: 1264
- ; silver salt: 1324, 1473
- , O₁¹⁸; β-lactone: 1874
- , 2-acetamido-2-cyano-3-phenyl-, -2-C¹⁴; ethyl ester: 226
- , 2-acetamido-3-(3,5-dibromo-4-hydroxyphenyl)-, -Br₂⁸²: 1150
- , 2-acetamido-3-hydroxy-, -3-C¹⁴: 199
- , 2-acetamido-3-phenyl-, -N¹⁵: 1751

- , 2-amino-, -N¹⁵: 1729
- , 3-amino-, -N¹⁵; and hydrochloride: 1740
- , 2-amino-3-chloro-; hydrochloride: 1970
- , 2-amino-3-hydroxy-, -1-C¹³-N¹⁵: 1742
- , 2-amino-3-hydroxy-, -3-C¹⁴: 169
- , methyl ester, hydrochloride: 172
- , 2-amino-3-hydroxy-, -3-C¹⁴₁-3-H²₁: 1294
- , 2-amino-3-hydroxy-, -2,3-H²-N¹⁵: 1743
- , 2-amino-3-hydroxy-, -N¹⁵: 1745
- , 2-amino-3-(*p*-hydroxyphenyl)-, -N¹⁵: 1729
- , 3-amino-2-mercapto-, -S³⁵; hydrochloride: 1952
- , 2-amino-3-phenyl-, -N¹⁵: 1729
- , L-: 1752
- , 2-amino-3-phenyl-H³: 1663
- , 2-benzamido-, -N¹⁵: 1733
- , 2-benzamido-3-chloro-; ethyl ester: 1967, 1970
- , methyl ester: 1970
- , 2-benzamido-3-hydroxy-: 1744
- , 2-benzamido-3-hydroxy-, -1-C¹³-N¹⁵; ethyl ester: 1742
- , 2-benzamido-3-hydroxy-, -3-C¹⁴₁-3-H²₁; ethyl ester: 1294
- , 2-benzamido-3-hydroxy-, -2,3-H²; ethyl ester: 1743
- , 2-benzamido-3-hydroxy-, -N¹⁵; ethyl ester: 1745
- , 3-benzyloxy-2-hydroxy-, -1-C¹⁴; ethyl ester, acetate: 938
- , 2-bromo-, D-: 1794
- , 2-bromo-, -Br⁸⁰: 1171
- , 2-bromo-, -Br⁸²: 1171
- , 2-bromo-, -2-C¹³: 141
- , 2-bromo-, -1-C¹⁴: 141, 164, 330
- , 2-bromo-, -2-C¹⁴: 138
- , 2-bromo-, -3-C¹⁴: 141
- , 2-bromo-, -2,3-H²: 1293
- , 3-bromo-, -1-C¹⁴: 168, 257, 260
- , ethyl ester: 258
- , 2,2-dimethyl-, -3-C¹⁴; *p*-bromophenacyl ester: 649
- , 3-chloro-; ethyl ester: 258
- , 3-chloro-, -1-C¹⁴: 731, 734
- , methyl ester: 731
- , 3-(chloroformyl)-; ethyl ester: 352, 353
- , methyl ester: 65
- , 2,3-dichloro-, -3-Cl³⁶: 1183
- , 3,3-diethoxy-; ethyl ester: 709
- , 3,3-diethoxy-2-methyl-; ethyl ester: 705
- , 2,3-dihydroxy-, -1-C¹⁴; diacetate, ethyl ester: 933
- , 2,2-dimethyl-, -3-C¹⁴; sodium salt (also see Pivalic acid): 649
- , 2,2-dimethyl-, -O¹⁸: 1880
- , O¹⁸-2,2-dimethyl-; ethyl ester: 1881
- , 2-ethoxalyl-; ethyl ester: 707
- , 3-ethylidene-2-hexyl-, -3-C¹⁴; β -lactone: 482
- , 3-(ethylthio)-, -1-C¹⁴: 158
- , 3-formyl-C¹⁴: 48
- , 3-glycl-2-C¹⁴: 353
- , 3-heptylidene-2-methyl-, -1-C¹⁴; β -lactone: 482
- , 2-hydroxy-, -2-C¹⁴: 139
- , 3-hydroxy-3,3-diphenyl-, -3-C¹⁴; ethyl ester: 838
- , 2-iodo-, -I¹²⁸: 1254
- , 2-(2-iodoacetamido)-3-phenyl-, -I¹³¹: 1205
- , H²-2-methoxy-: 1596
- , ethyl ester: 1643
- , 2-methyl-, -3-C¹³: 37
- , 2-methyl-, -1-C¹⁴: 95, 870
- , ethyl ester: 909
- , isobutyl ester: 875
- , sodium salt: 875
- , 2-methyl-, -2-C¹⁴: 37
- , 3-(methylthio)-: 972
- , 2-octanoyl-, -1-C¹⁴; ethyl ester: 482
- , 3-phenyl-, -2-C¹⁴: 885
- , 3-phenyl-, -3-C¹⁴: 76
- , 3-phenyl-, -2-H²: 1276
- , 3-phenyl-, -3-H²: 1457
- , 3-phthalimido-; ethyl ester: 1740
- , 3-phthalimido-, -N¹⁵; methyl ester: 1740
- , 3-*p*-tolyl-: 79
- Propionic acid-H²: 1261
- , -2-H²: 1266
- , 2-methyl-, -2-H²: 1312
- Propionic anhydride: 91, 1522
- Propionimide acid, 3-benzyloxy-2-hydroxy-, -1-C¹⁴; acetate, hydrochloride: 938
- , 3-(methylthio)-, -1-C¹⁴; hydrochloride, methyl ester: 629
- Propionitrile, -1-C¹³: 501, 805
- , -2-C¹³-3-C¹⁴: 141
- , -1-C¹⁴: 500
- , 2-amino-N¹⁵: 1733
- , 2-amino-3-(benzylthio)-2-methyl-, -1-C¹⁴: 208
- , 2,2'-azobis(2-methyl-, -1-C¹⁴): 487, 488
- , 2,2'-azobis(2-methyl-C¹⁴-, -3-C¹⁴): 488

- Propionitrile (*Continued*)
 —, 3-benzyloxy-2-hydroxy-, -1-C¹⁴; acetate: 938
 —, 2,3-di-4(or 5)-imidazolyl-, -1-C¹⁴; hydrogen oxalate: 528
 —, 3-(ethylthio)-, -1-C¹⁴: 159
 —, 2,2'-hydrazobis(2-methyl-, -1-C¹⁴): 487
 —, 2,2'-hydrazobis(2-methyl-C¹⁴-, -3-C¹⁴): 488
 —, 3-(methylthio)-, -1-C¹⁴: 629
p-Propionotoluidide, 2-acetamido-N¹⁵-3-phenyl-, L-: 1752
 Propionyl chloride: 164, 331, 1261
 —, -2-C¹³-3-C¹⁴: 141
 —, -1-C¹⁴: 378, 379, 481, 942
 —, 3-acetoxy-2-phenyl-, -2-C¹⁴: 551
 —, 3-carbethoxy-: 352, 353
 —, 3-carbomethoxy-: 65
 —, 2-methyl-, -2-H²: 1313
 Propiophenone: 193, 663
 —, -1-C¹⁴: 662
 —, -2-C¹⁴: 667, 836
 —, -2,3-H₂; and semicarbazone: 1518
 —, 2,3-dibromo-4'-methoxy-3-phenyl-, -1-C¹⁴: 643
 —, 1,2-dibromo-3-phenyl-, -1-C¹⁴: 670
 —, 3-dimethylamino-, -3-C¹⁴; hydrochloride: 672
 —, 2,3-epoxy-3- (*p*-methoxyphenyl)-, -1-C¹⁴: 671
 —, 2,3-epoxy-3-phenyl-, -1-C¹⁴: 669
 —, 4'-hydroxy-, -1-C¹⁴: 668
 —, 3-hydroxy-3-phenyl-3-phenyl-H₂-: 1342
 —, 2-oxa-, -1-O¹⁸: 1877
 —, 2-oxa-, -2-O¹⁸: 1875, 1877
 β-Propiothetin, dimethyl-C¹⁴-, hydrochloride: 972
 Propylamine, -1-C¹⁴; perchlorate: 500
 —, -3-C¹⁴: 51, 502
 —; hydrochloride: 502
 —, 3-chloro-*N,N*-dimethyl-: 515, 516
 Propylene glycol, -1-C¹⁴: 929
 Propylene oxide: 1907
 Propyne: 1434, 1436
 —, H²-, 1620
 —, -1-H²: 1427, 1434, 1435, 1436, 1483
 —, -3-H₂: 1436
 —, -3-H₂: 1436
 —, -1,3-H₂: 1436
 —, -3-H₂: 1436
 —, -1,3-H₂: 1436
 —, -H₂: 1427, 1435, 1436, 1496, 1513
 —, 1-chloro-, -H₂: 1497
 —, 3,3,3-trifluoro-, -H²: 1607
 Protocatechuic acid, -C¹⁴: 325
 Protocatechuy alcohol, α-(aminomethyl-C¹⁴): 949
 Provitamin A, C¹⁴-, 1113
 Pseudoionone, C¹⁴-, 651
 Pseudourea, 2-butyl-2-thio-, -S³⁵: 1933
 —, 2-ethyl-1-C¹⁴-2-thio-, picrate: 205
 —, 2-methyl-, -C¹⁴: 272
 —; hydrochloride: 272, 588
 —, 2-methyl-, -1,3-N¹⁵₂; and hydrochloride: 1754
 —, 2-methyl-C¹⁴-2-thio-, picrate: 199
 Pseudouric acid, -2-C¹⁴: 771
 —, -8-C¹⁴: 771
 —, -9-N¹⁴; and ammonium salt: 1851
 —, -1,3-N¹⁵₂; potassium salt: 1853
 6-Pteridinecarboxylic acid, 2-amino-4-hydroxy-, -C¹⁴: 308
 4,6-Pteridinediol, 2-amino-, -6,7-C¹⁴: 954, 955
 4,6,7-Pteridinetriol, 2-amino-, -6,7-C¹⁴: 954
 Purine, 6-acetamido-, -8-C¹⁴: 1043
 —, 6-amino-, -2-C¹⁴: 747
 —, 6-amino-, -2,8-H₂: 1676
 —, 6-amino-, -1,3-N¹⁵₂; sulfate: 1844
 —, 2-amino-6-hydroxy-, -8-H²: 1676
 —, 6-chloro-: 1939
 —, 2,6-diacetamido-, -2-C¹⁴: 1044
 —, 2,6-diamino-, -2-C¹³; sulfate: 757
 —, 2,6-diamino-, -2-C¹⁴: 1044
 —; sulfate: 757
 —, 2,6-diamino-, -1,2,3-N¹⁵₃; sulfate: 1847
 —, 6-mercapto-, -8-C¹⁴: 758
 —, 6-mercapto-, -S³⁵: 1939
 β-Purine, 2-acetamido-6-amino-9-β-D-ribofuranosyl-, -2-C¹⁴: 1045
 —, 6-acetamido-9-chloromercuri-, -8-C¹⁴: 1043
 —, 6-acetamido-9-(triacetyl-β-D-ribofuranosyl)-, -8-C¹⁴: 1043
 —, 6-amino-9-β-D-ribofuranosyl-, -8-C¹⁴: 1043
 —, 2,6-diacetamido-9-chloromercuri-, -2-C¹⁴: 1044
 —, 2,6-diacetamido-9-(triacetyl-β-D-ribofuranosyl)-, -2-C¹⁴: 1044
 —, 2,6-diamino-9-β-D-ribofuranosyl-, -2-C¹⁴: 1044
 2,6(1*H*, 3*H*)-Purinedione, -8-C¹⁴: 759
 —, -1,3-N¹⁵₂: 1850
 6-Purinethiol, -8-C¹⁴: 758
 —, -S³⁵: 1939
 8-Purinethiol, 2-amino-, -8-C¹⁴: 766
 —, 6-amino-, -8-C¹⁴: 753
 —; sulfate: 754
 2,6,8(1*H*, 3*H*, 9*H*)-Purinetriene, -2-C¹⁴: 773
 —, -6-C¹⁴: 770
 —, -9-N¹⁵₂: 1851
 —, -1,3-N¹⁵₂: 1853

- 2 Purinol, 6-amino-, -1,3- N_1^{15} : 1842
 6(1H)-Purione, -8- C^{14} : 759
 —, -1,3- N_1^{15} : 1840
 —, 2-amino-, -1,2,3- N_1^{15} ; sulfate: 1850
 Purrescine: 508
 —, C^{14} —: 86
 —, C_1^{14} —; hydrochloride: 656
 —, -1,4- C_2^{14} : 507
 —; dihydrochloride: 507
 —; reineckate: 507
 Pyran, tetrahydro-2- (tetrahydrofurfuryloxy)-: 899, 902, 913
 Pyran-2-carbonitrile, tetrahydro-2-methyl- C^{14} -6,6-dimethyl-: 355
 Pyran-2-carboxylic acid, tetrahydro-2-methyl- C^{14} -6,6-dimethyl-: 355
 4H-Pyran-2,6-dicarboxylic acid- H_2^2 , 4-oxo-, - H_2^2 : 1388
 4H-Pyran-4-one: 1387
 —, -2,6- H_2^2 : 1387
 —, -3,5- H_2^2 : 1388
 —, - H_2^2 : 1388
 —, 2,6-dimethyl-: 1388
 —, 2-(hydroxymethyl)-5-methoxy-: 697
 —, 5-methoxy-2-(methoxy- C^{14} -methyl)-: 697
 3-Pyrazolecarboxylic acid, H^2 -5-methyl-1-phenyl-: 1649
 2-Pyrazolin-5-one, 1-(*p*-bromophenyl)-4-(*p*-bromophenylazo)-, - Br_2^{82} : 1154
 3-Pyrazolin-5-one, 2-(*m*-chlorophenyl)-4-hexyl-3-hydroxy-, -4- C^{14} : 696
 —, 2-(*o*-chlorophenyl)-4-hexyl-3-hydroxy-, -4- C^{14} : 695
 —, 2-(*p*-chlorophenyl)-4-hexyl-3-hydroxy-, -4- C^{14} : 696
 —, 2,4-dihexyl-3-hydroxy-, -4- C^{14} : 696
 Pyrene, H^2 —: 1639
 —, - H_{10}^2 : 1447
 Pyribenzamine, C_1^{14} —; hydrochloride: 531
 Pyridine: 1081, 1386
 —; chromium trioxide complex: 538
 —, -2- H^2 : 1384
 —; dimercuric chloride complex, hydrochloride: 1385
 —, -3- H^2 ; and dimercuric chloride complex, hydrochloride: 1385
 —, -4- H^2 ; dimercuric chloride complex, hydrochloride: 1385
 —, - H_2^2 : 1385
 —, 3-acetyl-1- C^{13} —: 691
 —, 2-amino-: 530, 1945
 —, 4-amino-: 478
 —, 2-(benzylamino- α - C^{14})—: 530
 —, 2-[benzyl- α - C^{14} -(2-dimethylaminoethyl)aminol]-: 531
 —; dipicrate: 532
 —; hydrochloride: 531
 —; methiodide: 532
 —, 2-bromo-: 1384, 1385
 —, 3-bromo-: 392, 1385
 —, 4-bromo-: 477, 478, 479
 —; hydrochloride: 479
 —, 4-chloro-: 1385
 —, 3-iodo-: 1201
 —, 3-iodo-, - I^{131} : 1201
 —, 5,5,6-trichloro-2,3,4,5-tetrahydro-, -2,3,4- H_3^2 - N^{15} : 1768
 1(4H)-Pyridineacetic acid, 3,5-diiodo-4-oxo-, - I_2^{131} : 1209
 3-Pyridinecarboxylic acid, 6-hydroxy-, - N^{15} : 1767
 3,5-Pyridinedicarboxylic acid, H^2 -2,6-dimethyl-: 1648
 2,4(1H,3H)-Pyridinedione, 3,3-diethyl-, -6- C^{14} : 717
 —, 3,3-diethyl-5-(hydroxymethyl)-, -6- C^{14} : 717
 —, 3,3-diethyl-5-methyl-, -6- C^{14} : 718
 3-Pyridinepropionic acid, β -oxo-, - β - C^{13} ; ethyl ester: 691
 Pyridinium bromide, 1-(carbethoxymethyl)-3-iodo-, - I^{131} : 1201
 —, 1-(carboxymethyl)-3-iodo-, - I^{131} ; hydrazide: 1201
 Pyridinium chloride, 3-carbamoyl-1-methyl- H_3^2 —: 1526
 Pyridinium *p*-chlorobenzoate, 1-iodo-, - I^{128} : 1222
 Pyridinium iodide, 3-carbamoyl-1-methyl- H_3^2 —: 1526
 Pyridinium 2-naphthoate, 1-iodo-, - I^{128} : 1223
 Pyridinium *m*-nitrobenzoate, 1-iodo-, - I^{128} : 1223
 Pyridinium *p*-toluenesulfonate, 4-pentyl-: 73
 —, 4-pentyl-1-phenacyl- β - C^{14} —: 73
 2-Pyridinol, H^2 —: 1647
 —, - N^{15} : 1768
 3-Pyridinol, H^2 —: 1647
 4-Pyridinol, H^2 —: 1647
 2(1H)-Pyridone, - N^{15} : 1768, 1783
 4(1H)-Pyridone: 1209
 —, 3,5-diiodo-, - I_2^{131} : 1209, 1224
 Pyrimidine, 2-amino-: 1948
 —, 4-amino-5-(bromomethyl)-2-methyl; hydrobromide: 1118
 —, 2,4-diamino-, -1,2,3- N_1^{15} ; sulfate: 1808
 —, 4,6-diamino-5-formamido-; sulfate: 755
 —, 4,6-diamino-5-formamido-, -1- N^{15} : 1844
 —, 4,6-diamino-5-formamido- C^{14} —; sulfate: 755
 —, 4,6-diamino-5-phenylazo-, -4- C^{14} : 749, 751

Pyrimidine (*Continued*)

- , 4,6-diamino-5-phenylazo-, -1-N¹⁵:
1843, 1845
- , 4,6-diamino-5-thioformamido-, -1-N¹⁵: 1845
- , 2,4-dichloro-, -4-C¹⁴: 1040
- , 2,4-diethoxy-, -4-C¹⁴: 1040
- , 2,4,5,6-tetraamino-, -2-C¹⁴: 757
- ; sulfate: 757
- , 2,4,5,6-tetraamino-, -1,2,3-N¹⁵; sulfate: 1847
- , 2,4,6-triamino-, -2-C¹⁴: 756
- , 2,4,6-triamino-, -1,2,3-N¹⁵: 1846
- , 4,5,6-triamino: 753
- ; sulfate: 753, 755
- , 4,5,6-triamino-, -4-C¹⁴; sulfate: 749, 751, 776
- , 4,5,6-triamino-, -1-N¹⁵; sulfate: 1844, 1845
- , 4,5,6-triamino-2-methyl: 755
- , 2,4,6-triamino-5-nitroso-, -2-C¹⁴: 757
- , 2,4,6-triamino-5-nitroso-, -1,2,3-N¹⁵: 1846
- 4-Pyrimidineacetic acid, 1,2,3,6-tetrahydro-2,6-dioxo-, -2-C¹⁴: 358
- 4-Pyrimidinecarboxaldehyde, 1,2,3,6-tetrahydro-2,6-dioxo-, -6-C¹⁴; and diethyl acetal: 361
- , 1,2,3,6-tetrahydro-5-methyl-2,6-dioxo-, -2-C¹⁴; diethyl acetal: 363
- , 1,2,3,6-tetrahydro-5-methyl-6-oxo-2-thioxo; diethyl acetal: 362
- , 1,2,3,6-tetrahydro-6-oxo-2-thioxo-, -6-C¹⁴; diethyl acetal: 360
- 4-Pyrimidinecarboxylic acid, 1,2,3,6-tetrahydro-2,6-dioxo-, -2-C¹⁴: 358
- , 1,2,3,6-tetrahydro-2,6-dioxo-, -6-C¹⁴: 359
- , 1,2,3,6-tetrahydro-2,6-dioxo-, -3-N¹⁵: 1714
- , 1,2,3,6-tetrahydro-6-oxo-2-thioxo-, -S³⁵: 1973
- 2,4-Pyrimidinediol, 6-amino-(also see Uracil): 1808
- , 5,6-diamino: 759
- 2,4(1H,3H)-Pyrimidinedione, -2-C¹⁴ (also see Uracil): 701
- , -N¹⁵: 1802
- , 5,6-diamino-, -4-C¹⁴: 769
- , 5-methyl-, -1,3-N¹⁵₂: 1803
- 2,4,5,6(1H,3H)-Pyrimidinetetrone, -2-C¹⁴: 713
- , -N¹⁵: 1806, 1807
- 2-Pyrimidinethiol, 4,6-diamino-, -1-N¹⁵: 1841
- 4-Pyrimidinethiol, 5,6-diamino: 758
- 2-Pyrimidinol, 4,6-diamino-, -1-N¹⁵; hydrochloride: 1842
- ; sulfate: 1841, 1842
- , 4,6-diamino-5-nitroso-, -1-N¹⁵: 1841, 1842
- , 4,5,6-triamino-, -1-N¹⁵; and sulfate: 1842
- 4-Pyrimidinol, 6-amino-2-mercapto-, -1,3-N¹⁵₂: 1839
- , 6-amino-2-mercapto-5-nitroso-, -1,3-N¹⁵₂: 1839
- , 2,6-diamino-, -2-C¹⁴: 761
- , 2,6-diamino-, -6-C¹⁴: 762
- , 2,6-diamino-, -1,2,3-N¹⁵; and sulfate: 1849
- , 5,6-diamino: 759
- , 5,6-diamino-, -1,3-N¹⁵₂: hydrochloride: 1840
- , 5,6-diamino-2-mercapto-, -1,3-N¹⁵₂: 1839, 1840
- , 2,6-diamino-5-nitroso-, -2-C¹⁴: 761
- , 2,6-diamino-5-nitroso-, -6-C¹⁴: 762
- , 2,6-diamino-5-nitroso-, -1,2,3-N¹⁵: 1849
- , 2,5,6-triamino: 766, 954
- ; dihydrochloride: 765
- ; sulfate: 308
- , 2,5,6-triamino-, -2-C¹⁴: 312, 777
- ; hydrochloride: 310, 312
- ; sulfate: 761
- , 2,5,6-triamino-, -6-C¹⁴; sulfate: 763, 764, 776
- , 2,5,6-triamino-, -1,2,3-N¹⁵: 1849
- 2(1H)-Pyrimidinone, 4-amino-, -1,3-N¹⁵₂: 1807
- , 4-amino-1-β-D-ribosyl-, -4-C¹⁴: 1040
- , 4-ethoxy-1-(2,3,5-triacetyl-β-D-ribosyl)-, -4-C¹⁴: 1040
- 4(3H)-Pyrimidinone, 6-ethyl-2-mercapto-, -S³⁵: 1975
- , 2-mercapto-6-methyl-, -S³⁵: 1974
- , 2-mercapto-6-propyl-, -S³⁵: 1975
- Pyrocatechol: 721
- , 4-(2-aminoethyl-1-C¹⁴); hydrochloride: 521
- , 4-(2-aminoethyl-2-C¹⁴); hydrochloride: 521
- Pyrogallol, H²: 1653
- Pyrophosphoric acid, -P³²; calcium salt: 1915
- Pyrrole, H²: 1647
- , -1-H²: 1382
- , -2,3,4,5-H²₄: 1383
- , -H²₃: 1382
- , H²-1-methyl: 1647
- , 1-potassium: 1382

2-Pyrrolidinecarboxylic acid, -C¹⁴: 240
 —, -3,4,5-H₃-N¹⁵: 1768
 —, -N¹⁵: 1770
 —, 4-hydroxy-, -2-C¹⁴: 242
 —, 4-hydroxy-, -N¹⁵: 1771
 Pyruvaldehyde, -1,3-C₂¹⁴: 467
 —, -1,3-C₁¹⁴: 143
 —, C¹⁴-hydroxy-: 620
 Pyruvamide, -2,3-C₂¹³: 346
 —, -1-C¹⁴: 343, 344
 —, -2-C¹⁴: 344, 346, 475
 —, -3-C¹⁴: 346, 348
 —; phenylhydrazine: 346
 —, -2,3-C₂¹⁴: 346
 Pyruvic acid: 1293, 1574, 1577, 1729
 —; 2,4-dinitrophenylhydrazine:
 1574
 —, -1-C¹³; butyl ester: 342
 —; sodium salt: 342
 —, -2,3-C₂¹³: 346
 —; butyl ester: 343
 —; sodium salt: 343
 —, -1-C¹⁴: 343
 —, -2-C¹⁴: 47, 115, 141, 302, 344,
 346, 832
 —; 2,4-dinitrophenylhydrazine: 345
 —; ethyl ester: 345
 —; phenylhydrazine: 302
 —; potassium salt: 345
 —; sodium salt: 345
 —, -3-C¹⁴: 346, 347, 348
 —; 2,4-dinitrophenylhydrazine: 348
 —; methyl ester, phenylhydrazine:
 782
 —; phenylhydrazine: 346, 782
 —; potassium salt: 347
 —, -2,3-C₂¹⁴: 346
 —, benzylidene-: 1763
 —, bromo-, -2-C¹⁴: 346
 —, hydroxy-, -2-C¹⁴: 346
 —, (*p*-hydroxyphenyl)-: 1729
 —, phenyl-: 1729
 —, phenyl-, -3-C¹⁴: 348
 Pyruvitrile, -2,3-C₂¹³: 346
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 —, 2-amino-5-guanidino-2,3-N¹⁵₂; and hydrochloride: 1755
 —, 2-amino-4-methyl-, -1-C¹⁴; L-: 212
 —, 2-amino-4-methyl-, -3,4-H₂: 1296
 —, 2-amino-4-methyl-, -2,3,4-H₃-N¹⁵: 1737
 —; D- and L-: 1738
 —, 5-amino-4-oxo-, -5-C¹⁴; hydrochloride: 353
 —, 5-amino-4-oxo-, -N¹⁵; hydrochloride: 1750
 —, 2-benzoyl-, -3-H₂; ethyl ester: 1522
 —, 5-bromo-, -1-C¹⁴; and methyl ester: 239
 —, 2-bromo-4-methyl-, -1-C¹⁴: 216
 —, 2-bromo-4-methyl-, -2-C¹⁴: 214
 —, 2-bromo-4-methyl-, -2,3,4-H₃; ethyl ester: 1737
 —, 2-bromo-5-(*m*-nitrobenzamido)-: methyl ester: 1777, 1780

—, 2-bromo-5-(*m*-nitrobenzamido- N^{15})-: 1783
 —, 2-bromo-5-phthalimido-, -1- C^{14} : 240
 —, 2-carboxy-2,5-dichloro-4-hydroxy-, -2- C^{14} ; ethyl ester, γ -lactone: 242
 —, 2-carboxy-5-chloro-4-hydroxy-, -2- C^{14} ; ethyl ester, γ -lactone: 242
 —, 2-carboxy-2,5-dichloro-4-hydroxy-, -2- C^{14} ; γ -lactone: 242
 —, 5-chloro-4-oxo-; methyl ester: 1750, 1751
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 —, 2,5-diamino-, -2- C^{14} ; dipicrate: 281
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 —, 2,5-diamino-4-oxo-; L-: 1773
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 —, 2,5-dichloro-4-hydroxy-; γ -lactone: 1772
 —, 2,5-dichloro-4-hydroxy-, -2- C^{14} ; γ -lactone: 242, 243
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 —, 2-ethoxycarbonyl-2,5-diphtalimido-, -2- N^{15} ; ethyl ester: 1780, 1782
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 —, 2-ethyl-1- C^{14} -2-methyl-3-oxo-; ethyl ester: 53
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 —, 4-hydroxy-, - O^{18} ; γ -lactone: 1874
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 —, 4-methyl-, -1- C^{14} : 96
 —; sodium salt: 216
 —, 4-methyl-, -2- C^{14} : 43, 214
 —; sodium salt: 214
 —, 2-methyl-3-oxo-; ethyl ester: 53, 54
 —, 5-(*m*-nitrobenzamido- N^{15})-: 1783
 —, 5-(*m*-nitrobenzamido)-2-(phthalimido- N^{15})-; methyl ester: 1777
 —, 3-oxo-; ethyl ester: 1975
 —, 4-oxo-5-phthalimido-, - N^{15} ; methyl ester: 1750
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 —; methyl ester: 239
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 —, 4-dimethylamino-2,2-diphenyl-: 653
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 —, -4,4'- C^{14} ; hydrochloride: 196
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 —, *N*-acetyl-3-mercapto-, - S^{35} : 1985
 —, *N*-chloroacetyl-, -4- C^{13} : 195
 —, 3-mercapto-, - S^{35} ; hydrochloride: 1986
 —, H^2 -phenylacetyl-L-alanyl-; D-: 1589
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 —; butyrate: 1136
 —; 3,5-dinitrobenzoate: 1135
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—; 2-diethylaminoethyl ester, metho-
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—, -1,3-N¹⁵; and silver salt: 1850

—, 1-methyl-C¹⁴-3,7-dimethyl-: 767

—, 7-methyl-C¹⁴-1,3-dimethyl-: 768

Xanthopterin, -6,7-C¹⁴: 954

—, α -dihydro-: 955

—, β -dihydro-: 955

—, dihydro-, -6,7-C¹⁴: 955

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o-Xylene, α, α' -dibromo-: 458

—, α, α' -dibromo-, - α -C¹⁴: 459

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2,6-Xylenol: 734

—, -4-H²: 1527

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—, 4-allyl-3-C¹⁴_: 735

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3,4-Xylenol, H²_: 1654

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dene-; D-: 1000, 1002, 1003,
1005

—, 5-aldo-1,2-O-isopropylidene-, -1-
C¹⁴; D-: 987

—, 5-aldo-1,2-O-isopropylidene-, -2-
C¹⁴; D-: 989

—, 1,2-O-isopropylidene-, -1-C¹⁴; D-:
987

Xylonic acid, -1-C¹⁴; D-, and lead salt:
984

—; D-, cadmium salt and cadmium
bromide double salt: 989

—; D-, γ -lactone: 984, 989

Xylose, -1-C¹⁴; D-: 987, 988

—; D- α _: 984

—, -2-C¹⁴; D-: 989

—, -5-C¹⁴; D-: 996

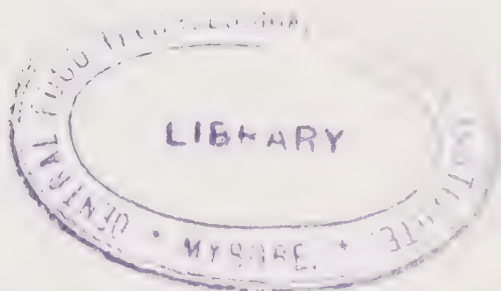
—, O¹⁸_: 1895

—, 2,4-O-furfurylidene-, -5-C¹⁴; D-:
996

Xylosone; L-: 1124

Z

Zinc, diethyl-: 1403



Organic synthesis.



